HOW CROPS GROW

A Century Later

HOW CROPS GROW

A Century Later

A series of lectures to celebrate
the Centennial of How Crops Grow by
Samuel W. Johnson—1868

Delivered September 17, 1968—May 19, 1969

Edited by Peter R. Day

Bulletin 708 July 1969

The Connecticut Agricultural Experiment Station
New Haven, Connecticut

The lectures commemorating the 100th anniversary of *How Crops Grow*, and their publication in this Bulletin, were made possible by the Lockwood Trust, an endowment of the Station.

MANUFACTURED IN THE UNITED STATES OF AMERICA BY
UNITED PRINTING SERVICES, INC.
NEW HAVEN, CONN.

Preface

Early in 1968 invitations were extended to ten distinguished scientists to help celebrate the centenary of the publication of the book *How Crops Grow* by Samuel W. Johnson. This book profoundly influenced the development of agriculture in the United States. It appeared seven years before the General Assembly of Connecticut established this Station, the first in America. Professor Johnson was director of the Station from 1877 to 1900. We thought it appropriate to consider how the subject of the book has developed during the intervening 100 years. During this time the growth of crops has concerned plant physiologists, biochemists, soil chemists, geneticists, plant pathologists, entomologists and climatologists. Most of these disciplines had not been born or were in their infancy in 1868. Indeed Mendel had published his researches just two years before but Johnson, and most of his contemporaries, were either unaware of this or overlooked its significance. In choosing our speakers and their subjects we were guided by the book itself and by what is relevant to current agricultural science. A book of this small size can only take a sampling of all that is known of how crops grow in 1968. Each of the scientists we invited is distinguished as a leader in his field and each was asked to bridge the gap between Johnson's time and ours in a public lecture at the Station. We suggested the titles, but this was our only attempt to fit our speakers to a mold.

What better way can there be to appreciate the growth, the exciting developments, and the consequences of what has happened in these areas of vital interest to us at this Experiment Station and other research institutions? We believe that these lectures deserve a wider audience and so present them in this book.

Peter R. Day, *Editor*
July 1969

Contents

The Contributors

Dr. J. G. Horsfall. Head of Plant Pathology and Botany from 1939 to 1948 and, since 1948, Director of The Connecticut Agricultural Experiment Station. Dr. Horsfall came to New Haven by way of the University of Arkansas and Cornell University. A member of the National Academy of Sciences, his research has included the development of new fungicides and chemotherapeutants.

Dr. H. B. Vickery. Chief biochemist at The Connecticut Agricultural Experiment Station from 1928 to 1963, presently Biochemist Emeritus, and member of the National Academy of Sciences, formerly President of the American Society of Biological Chemists. Dr. Vickery's interests include the chemistry of amino acids, proteins, plant organic acids, and the history of protein chemistry.

Dr. F. C. Steward. Alexander Professor of Biology and director of the Laboratory of Cell Physiology and Growth, Cornell University. Dr. Steward edited a series of monographs on plant physiology, has carried out research on salt absorption, respiration, nitrogen metabolism and growth, and morphogenesis in plants.

Dr. P. J. Kramer. James B. Duke Professor of Botany at Duke University, Durham, North Carolina since 1954 and member of the National Academy of Sciences. Author of the classic *Plant and Soil Water Relationships* (1949), Dr. Kramer has worked on water and salt absorption by plants and effects of environment on plant growth.

Dr. Martin Gibbs. Professor and past chairman of the Biology Department at Brandeis University and, since 1963, Editor-in-chief of *Plant Physiology.* Formerly Professor of Biochemistry at Cornell 1956-1964. Research on carbon metabolism in photosynthesis and respiration in plants.

Dr. O. E. Nelson. Professor of Genetics in Department of Botany and Plant Pathology at Purdue University. Dr. Nelson obtained his Ph.D. at Yale while a student of the late Dr. D. F. Jones of our Station. In September 1969 Dr. Nelson joined the Genetics Department of the University of Wisconsin. His research has been largely concerned with the physiological genetics of corn.

Dr. S. B. Hendricks. Chief chemist of the U. S. Bureau of Plant Industry since 1948. Member of the National Academy of Sciences and recipient of numerous awards. Dr. Hendricks is an avid mountaineer,

having made the third ascent of Mount McKinley. His research interests include chemical structure, soil structure, plant nutrition, and photoperiodism.

Dr. H. J. Evans. Professor of Plant Physiology at Oregon State University since 1961. A native of Kentucky, he received his Ph.D. at Rutgers University and was for a time on the faculty of North Carolina State University. His research interests have been nitrate reduction, the activity of cations, such as cobalt and potassium, on plant enzymes and more recently biological nitrogen fixation.

Dr. Arthur Kelman. Professor and chairman of the Department of Plant Pathology of the University of Wisconsin since 1965. Dr. Kelman was born in Rhode Island, spent one summer here and received his Ph.D. at North Carolina State University in 1947 where he later became W. N. Reynolds distinguished professor. His interests have centered on the factors influencing virulence and resistance to bacterial plant pathogens.

Dr. P. L. Adkisson. Professor of Entomology at Texas A. & M. University since 1965. A native of Arkansas, Dr. Adkisson received his Ph.D. at Kansas State University. His research has been on cotton insect control and the photoperiodic control of insect diapause.

Dr. L. J. Haworth. Director of The National Science Foundation from 1963 to 1969. Dr. Haworth, a native of Michigan, is a physicist. A member of the National Academy of Sciences, his many distinctions include service on two Presidential Commissions and as Director of Brookhaven National Laboratory from 1948 to 1961. Dr. Haworth returned to Brookhaven in 1969 as Special Consultant to the President of Associated Universities, Inc. His research interests cover crystal structure, acoustic instruments, and nuclear reactor and accelerator design.

How Johnson Related Science to Society

James G. Horsfall

Those who cannot remember the past
are condemned to repeat it. SANTAYANA

Johnson's work is history. His methods for relating science to society written during the sixties of the last century display an astonishing prescience. Even so, they are being rediscovered and urgently advocated in 1969, as shown in the closing paragraphs of this essay.

We modern men of science writing in this book HOW CROPS GROW, 1968, can add no whit to the stature of Samuel W. Johnson, but we can pay our respects by celebrating the centennial of his book and by showing how his thoughts which he condensed so aptly into three words, **How Crops Grow,** remain cogent today.

The President of the American Institute of Biological Sciences, writing in this year of 1969, reminds us that we never outgrow the need to relate science to society.

> Thoughtful observers of the national scene have been aware for some time that the relationship between the scientific fraternity and the public has been steadily deteriorating, and nowhere is this more apparent than in the halls of Congress. Biologists can respond to this disaffection in a number of ways: they can sulk; they can continue to talk to each other and to marvel at the ingratitude of the human race; they can be raucous in denunciation of their leaders; or they can recognize their responsibilities as citizens as well as scientists, and take the initiative in bridging the gap. We believe that the latter course is the only tenable one. The National Biological Congresses represent the first practical step by biologists to marshal their skills for the common good and, at the same time, to communicate a sense of mission and involvement to the public.

The Stations themselves, and the Extension services of the land-grant universities, have long communicated "a sense of mission and involvement to the public." However innovative the steps taken by the National Biological Congresses, they in fact follow in the footsteps of Johnson, his contemporaries, and his successors.

In this essay I shall explore how Johnson marshalled the skills of biologists for the common good. It took him nearly 25 years.

What Others Have Said of Johnson

During the last 100 years, people have spoken well of Johnson and his writings. Herewith is a sampling, going back from the current year.

In April, 1969, the *Journal of Chemical Education* said, ". . . Johnson educated under Liebig, had enormous influence not only on the development of the Sheffield Scientific School at Yale, but on the creation of the first Agricultural Experiment Station in the U.S., that in Connecticut."

In his book published 20 years ago, E. J. Dies listed Johnson as one of 17 "Titans of the Soil, the great builders of American agriculture." Said he, "Of all the men who struggled to build an agricultural empire, none worked with nobler effort than Johnson."

When Johnson died in 1909, *Science* said, "In the death of Samuel William Johnson, the chemists of America have lost one more from that small band, who two generations ago, undertook to extend and develop the beginnings which had been made to establish laboratories for instruction in chemistry and to apply this science to the industries of the country."

The *Experiment Station Record* remarked in 1909 that ". . . his name will be intimately linked with the early history and the development of agricultural science in this country. He was a pioneer, a leader of thought, the disciple of a new idea in science."

A still earlier appraisal of Johnson's book was made at a meeting of agricultural educators in Chicago in 1871 although it remained unpublished until 1967. Mr. Flagg, of the University of Illinois, stated, "I think . . . that Prof. Johnson, in his "How Crops Grow," and "How Crops Feed," is doing more to advance agricultural interests than any man in the United States."

And finally the powerful impact of Johnson's book was apparent as soon as it was printed. Liebig expressed his approval by arranging for its German translation. Lawes, the founder of the Rothamsted Experimental Station, wrote Johnson: ". . . it is the best summary of information on the subject which has yet been published."

This book and his other writings exerted a powerful influence because Johnson was an extraordinarily penetrating and prescient thinker. And he was persuasive.

The Philosophy of a Yale Sophomore

As a college sophomore in 1851 he wrote an article for the *Albany Cultivator* proposing that "farmers set up an agricultural institute . . . to afford greater facilities for experimental agriculture . . . and to provide men and means for striking out into the path of discovery, for increasing as well as diffusing knowledge . . . [The Institute] should possess a legal incorporation . . . located near an academy . . ."

It is all there—science should serve society (applied science)—provide men and means—the path of discovery (basic science)—increase as well as diffuse knowledge (produce and publish). Thus Johnson recognized the need for applied as well as basic science and that scientists should produce and publish their work.

Essentially all of these thoughts showed up in his book published 18 years later. He says (p. iv), "In preparing the ensuing pages the writer has kept his eye steadily fixed upon the practical aspects of the subject. . . . He would earnestly invite young men . . . who are conscious of the power of investigation to enter the fields of agricultural science, now white with the harvest for which the reapers are all too few . . ."

The essay of Johnson, the Yale sophomore, sets forth the essential features of The Connecticut Agricultural Experiment Station as it exists today, 118 years later—a corporation governed by a Board of Control, located near an Academy (Yale) but separate from it, devoted to "making a regular business of discovery" and to putting the discoveries to work for the food supply.

The Odds He Struggled Against

Johnson found the going rough. It was not easy in the eighteen fifties to sell science to society, "book learning" to farmers and legislators.

He struggled under enormous odds to give life and strength to his proposal to put science to work. His salary came from Yale College. This was hardly a farm school. In 1960, *Scientific American* published this excerpt from its issue of a century earlier: "To see Yale College stepping out from among the mists of antiquity and the graves of dead languages, and 'taking up the shovel and the hoe,' is certainly one of the signs of the times. She made her debut on this new stage on the 1st day of February having secured the services of 25 leading agriculturists to sustain her in this first effort." Johnson helped persuade Yale College to do this, but he found it hard to keep Yale College convinced on science, to say nothing of agriculture.

Having more or less convinced Yale College, he then had to convince hard headed farmers and legislators. One farmer testified at the 1873 hearing on the bill in the Connecticut General Assembly, to establish the Experiment Station. "I tell you go slow," he said, "You are hatching an egg that will certainly make this state a lot of trouble." One could hardly blame the legislators for refusing to pass the bill the first time it was introduced.

Despite such formidable obstacles at Yale and in conservative Connecticut, Johnson did succeed. He did establish the concept that science could be made useful to society, and that society would pay for it. Fifty-three experiment stations in America proclaim it today and so do the distinguished laboratories of the Bell System, General Electric, and others.

The Persuader Was Fertilizer Analysis

Johnson was a very shrewd tactician. He knew that science would have to do something for society and do it fast, if his idea were not to die. Accordingly, his first effort was to analyze fertilizer to prevent fraud. Everybody knew that plants need fertilizer, but nobody could distinguish the value of Quinnipiac River mud from Peruvian guano in a bag labelled, "fertilizer." Johnson's chemistry could. America was as materialistic then as now and it could and did appreciate this help from science. It still does.

Other Experiment Stations in the other states quickly followed Johnson's lead. For example, a report of the first 60 years of the North Carolina Station says, "In 1876 Dr. Battle [President of the University] made a visit to Connecticut where he conferred with . . . the Director of the first Agricultural Experiment Station in the United States. He returned with enthusiasm . . . and strongly advocated the establishment of a similar institution in North Carolina."

They analyzed fertilizer, too, and thereby became the second legal entity to be called an Agricultural Experiment Station.

Industry Followed Later

It is interesting and probably significant that agriculture was the area where science was put to work first. It was 25 years before the General Electric Co. established a laboratory where science could work for it. The DuPont company came shortly thereafter. The agricultural background of the industrial laboratory is reflected in the name of the DuPont laboratory. They called it then and still do call it their "Experimental Station."

Forty years more had to pass before the Government organized science to work for the art of defense. Vannevar Bush and James Conant set up numerous "Experimental Stations" during the war; The Radiation Laboratory, The Manhattan District, Los Alamos, Oak Ridge, etc. They put science to work for the military 65 years after Johnson put it to work for agriculture.

Johnson showed how to feed the nation with fewer farmers. Bush and Conant showed how to protect the nation with fewer soldiers.

Professionals from Amateurs

When Johnson's book was published, scientists were amateurs. The science of Mendel was supported by the Church, that of Darwin by the Wedgewood pottery. When Johnson persuaded society to pay for science from public funds, science became a professional pursuit. Hereafter, scientists would earn their own keep from their contributions to society. Scientists would live off science and not science off scientists.

Of course, a professional has fewer degrees of freedom than an ama-

teur. He has fewer degrees of freedom when supported by public funds than when supported by his father's funds or some other father's funds.

Agricultural scientists soon stopped playing by amateur rules. They could see which side of their bread had the butter. Johnson had told them.

The Pendulum Overswings

In some cases, however, the pendulum swung too far, as it often does. In many quarters the purely pragmatic side of agricultural science was overemphasized. In their 50th anniversary reports numerous experiment stations stated that their function ". . . is to apply to the agriculture of [their state] the recent advances of science." Little was said of the need for theoretical research that makes the recent advances in science. This part of Johnson's dictum was often neglected.

Johnson's Concern for the Theoretical

The irony of all this is that Johnson did not intend that the pendulum should overswing. Johnson wanted basic research as much as applied. After all he was elected to the National Academy of Sciences when he was 36 years old. Once he got his fertilizer analyses underway, his next move was to appoint T. B. Osborne, who distinguished himself as a theoretical biochemist.

Johnson foresaw or anticipated the controversy over basic and applied research that continues to this day. He encompassed his whole view of this matter in the three words that title his book. Johnson said, in effect, let us work on "How Crops Grow." The middle word, crops, showed his concern for the food supply of an industrial society. How they grow showed his concern for the basic aspects of biology. Overemphasis on the practical would rephrase Johnson's title to "How to grow crops." Johnson sensed that if the scientists know how crops grow, they could and would provide the farmer with the information for growing vastly better crops, so that society would be plentifully fed.

His remarkable foresight in this area shows through in an article written from Liebig's laboratory in Munich in 1855. He called it, "On theory and practice"—basic and applied if you please!

> Every farmer has a theory. Every sane man performs all of his business in accordance with some rules which satisfy his mind . . .
>
> The farmer may affect to despise theory, and claim that he is purely a practical man; but he is mistaken. For to suppose that a man can carry out the manifold affairs of a farm, through a whole year and through many years, without some mental basis for his operations, is sheer nonsense. This mental basis is theory, and to take pride "in freedom from theory," is to boast of inability to think.
>
> Practice and theory ought to go together . . .
>
> Rational Agriculture is the result of such a union.

> To prevent confusion, and enable our language to convey the idea of cost, included or excluded, I propose to the Agricultural press, the constant use of the term AGRICULTURAL SCIENCE, to signify the scientific theory—the abstract doctrines on which agriculture is based, independent of profit or economy; and SCIENTIFIC AGRICULTURE to denote the practical theory, the applied doctrines of agriculture, founded on natural laws, taken in connection with economical considerations. The former can be cultivated in the study and laboratory; the latter must be learned in the field and sustain the ordeal of practice. The former is the intangible offspring of mind; the latter is the mental brood made incarnate. The one is satisfaction to the philosophical intellect; the other is profit to the practical purse.
>
> Agriculture will flourish from that day, when practical men shall be philosophical enough to appreciate the philosopher's thoughts; and philosophers practical enough to calculate the farmer's profits.
>
> *Munich, March, 1855.*

Our squabbles in the sixties of this century about basic and applied science reveal that many scientists want to have their cake and eat it too. They want to use amateur's rules in a professional game. They want their funds to pour from the public purse, but they are desperately afraid of their image at home in academe if they show any interest in subjects of practical importance to society. As *Time* magazine said recently, there are no Nobel prizes for a scientist who discovers how to rid a city of rats. Perhaps the new generation of scientists will see to it that there are.

Our failure in the sixties to follow Johnson's dictum that "Practice and theory ought to go together" led us into trouble in Congress and to the remarks of the President of the American Institute of Biological Sciences set out in the opening paragraphs above.

Senator Allott of Colorado "told it like it is" in 1968 when he wrote in *Science*, "For some time I have been warning members of the scientific community that unless some adequate means are developed so that the taxpayers and their elected representative know what they are 'buying' with their research dollars, a reaction would set in one day which would cause a severe cutback in funds allocated for research."

Playing as amateur scientists we found it hard to listen. Many could not hear the signal for the noise. Some have listened with an acute ear. For example, Kenneth Thimann, a distinguished botanist recently from Harvard, wrote an editorial for *Science*, May 30, 1969, entitled "Science as an instrument of service" and says, "In the past we science teachers have stressed the fascination of science, the unity of science, or the power of the scientific method, it is time now to stress the role of science as an instrument of service. . . ."

So spake Johnson a century ago.

Samuel W. Johnson and *How Crops Grow*

H. B. Vickery

This series of lectures celebrates the one-hundredth anniversary of the publication of a little textbook on agricultural chemistry. It was written by Samuel William Johnson, professor of analytical and agricultural chemistry in the Sheffield Scientific School of Yale University, and from 1877 until he retired in 1900, the Director of The Connecticut Agricultural Experiment Station. Published in 1868, it was immediately used in the agricultural colleges in this country, and within a few years, in translations into at least five other languages, it spread its influence over agricultural schools throughout the world. It became the fundamental textbook in the subject for at least forty years.

Johnson wrote it, as he says in the Preface, as a result of the studies he had made in preparing the lectures to his annual classes at the Scientific School. He sought to present all of the facts which would "serve the student of agriculture for thoroughly preparing himself to comprehend the whole subject of vegetable nutrition . . . in the hope that a company of capable investigators may be raised up, from whose efforts the science and the art may receive new and continual impulses."

The application of science to agriculture during the past century illustrates how truly Johnson judged the conditions of his time. My assignment is to discuss some aspects of its history, and I shall begin by talking about Johnson himself, for I am afraid that to most of the present generation he is merely a name, not a personality.

Johnson was the third son and fourth child of Abner A. Johnson, a merchant and tavern keeper in Kingsboro, New York who, in 1834 when the boy was four years old, gave up his business and bought a large farm in Deer River, a few miles from Lowville. Here he became a prominent farmer of the region, raising a family of eight children all of whom were educated at the Lowville Academy. It was here that young Johnson's interest in chemistry was first aroused by his teacher who, at his graduation in 1846, gave him a copy of the most recent (1844) edition of Fresenius' textbook on chemical analysis translated by J. Lloyd Bullock, a student of Liebig and a colleague of its author. This book is in the Osborne collection in the library at the Station. A statement on the flyleaf in Johnson's handwriting tells how he obtained it, and goes on to say that he had prepared the reagents and had worked

through much of the course in qualitative analysis given in the text. The condition of many of the pages, with spatters of precipitates and of occasional drops of acid, is clear evidence of the boy's industry.

Johnson shared with his older brothers in the work of the farm and taught elementary school during two winters after graduation from Lowville. His efforts to persuade his father that his future lay in a life devoted to science met with only partial understanding. Medicine or the law were to the father the only promising professions aside from farming, and matters evidently came to a head in the fall of 1848, for Johnson applied for and obtained a position as instructor at a preparatory school in Flushing, Long Island, at a salary of $20 a month with room and board. Here he proposed to demonstrate that he could support himself by teaching and could also continue his studies. Further education was, however, obviously essential and, after correspondence with Professor Horsford at Harvard which indicated that the costs of going there were well beyond his means, he visited New Haven in the fall of 1849 for an interview with Professor Norton.

John Pitkin Norton (1822-1852), son of a prominent and wealthy farmer of Farmington, Conn. had decided at age 18 that he wished to become a farmer and manage his father's property. The father, under the influence of articles in the *Albany Cultivator* on natural science as applied in Europe to farming, responded by sending the boy to Yale to study chemistry under Professor Benjamin Silliman who, at that time, accepted a few special students for private instruction. During Silliman's frequent absences giving public lectures, these students were supervised by his son and assistant Benjamin Silliman, Jr. In 1843, Silliman arranged for Norton to go to Edinburgh to study under James F. W. Johnston, the chemist of the Scottish Agricultural Chemistry Association who was conducting what was, in effect, an agricultural experiment station, the first of its kind in the world. It was supported by public funds, and its chemist had the duty not only to carry out chemical analyses of agricultural products, but also, on request, to address farmers' meetings on scientific topics. Norton spent nearly two years with Johnston, not only learning chemistry, but also accompanying his chief to meetings all over Scotland.

Norton returned to Yale in 1846 with the ambition to teach scientific agriculture. Meanwhile, the Sillimans had prevailed upon the Yale Corporation to set up a department in which advanced students could obtain laboratory instruction in science. With the help of a grant of five thousand dollars to Yale from Norton's father, a chair of agricultural chemistry was established, and young Norton was appointed professor, a position he occupied until his death in 1852. A second chair of practical chemistry (i.e. analytical chemistry) was also provided for, and Benjamin Silliman, Jr., received the appointment. Norton spent the next nine months with G. J. Mulder in Utrecht learning to analyze his preparations of seed proteins made when studying with Johnston and, in 1847, public announcement was made of the new School of Applied

Chemistry, a department of Yale College which ultimately developed separately into the Graduate School and the Sheffield Scientific School of the University.

Johnson returned to Flushing from his interview with Professor Norton full of enthusiasm for the tiny school at Yale and, in January 1850, relying wholly upon his savings of less than $200 he came to New Haven to begin his studies of analytical chemistry. In the fall he finally convinced his father that there might be a future for him in chemistry and was given an allowance sufficient to support him, although rigid economy was necessary. Eighteen months at Yale under the influence of Norton convinced Johnson that further study in Germany was essential if he was to equip himself for a career in chemistry, but the problem of ways and means remained. Accordingly, in the summer of 1851 he applied for and received the appointment of Professor of Chemistry, Natural Philosophy and the Principles of Agriculture in the State Normal School in Albany. The salary was $800 which to him at the time seemed munificent, and he was able to save enough to return to the New Haven laboratory for another year with only moderate assistance from his father. However, the problem of the means for foreign study was still unsolved.

It is perhaps significant that Johnson senior, like Norton's father, also read the *Albany Cultivator*. At all events, at this point he finally conceded the issue. For five years the young man had demonstrated his ability as a student and as a teacher, the two older brothers had each been given farms on reaching age 21, and the third son obviously merited equal treatment. Accordingly the father set aside property equal to that given to the older boys and gave his consent to the trip abroad. Johnson was thus able to spend a year in Erdmann's laboratory in Leipzig studying analytical chemistry and the greater part of a second year with Liebig in the newly established laboratory in Munich. He also traveled widely, meeting many prominent chemists and returned to New Haven in the fall of 1855 to an appointment as first assistant in the analytical laboratory. The following year he was appointed Professor of Analytical Chemistry in what was now called the Yale Scientific School, forming a connection he retained for the rest of his life. It is an interesting sidelight that the appointment to the professorship from Yale College carried with it an honorary Master of Arts degree, the only formal college degree that Johnson ever held. He had throughout his training period specialized in chemistry; he had not prepared himself for the Bachelor of Philosophy degree offered by the Yale school in the early 50's, nor had he troubled to take the examinations for the Doctor's degree at either of the German universities he attended.

What has been said omits reference, however, to Johnson's other and equally important activity. While still a boy of 17, he had sent a brief essay "On Fixing Ammonia" to the *Albany Cultivator* in which he pointed out the valuable service which chemical science could render to agriculture. During the early years at Yale and his student period in

Germany he wrote about thirty articles, mostly for Luther Tucker, the publisher of the *Country Gentleman*, on the composition of fertilizers, on agriculture as practiced in Germany, and on agricultural education; included also were translations of several publications by Liebig and by Wolff. He thus carried on the tradition established by Norton who had made frequent contributions to the *Albany Cultivator*. During the same period, there were about twenty contributions to scientific journals, mostly on analytical methods or on the analysis of minerals. Thus, on his return from Europe in 1855, Johnson was widely known, not only to the scientific fraternity, but also to the farm public. He was at once invited to lecture before the New York State Agricultural Society in Albany and, the following year, before the Connecticut State Agricultural Society. At this meeting he was elected to be their official chemist.

Johnson had obtained an education: by strength of character, ability, and plain hard work he had become a skilled analytical chemist, had acquired a broad knowledge of agricultural practices both in this country and in Europe, and, by reading practically everything published on the subject in Germany, France, and Britain, had made himself a complete master of current theory in chemistry and biology as applied to agriculture. In addition, he had formed lasting friendships with many of the leaders of science in Europe, such as Liebig, Wolff and Knop, and especially with the group of young Americans who were his contemporaries as students in the German universities. These included such men as William H. Brewer and George J. Brush, later of the Sheffield Scientific School, F. H. Storer, later of the Bussey Institute, M. C. Weld, a fellow student at Yale and his roommate in Germany who became editor of the *Homestead* published in Hartford, Evan Pugh, later the first president of what became the University of Pennsylvania State College, Peter Collier, later Director of the New York Agricultural Experiment Station, and many others.

What, then, were the problems that Johnson faced as a young man with the mission to render scientific service to the farmer. The first was the widespread ignorance of chemistry and of what chemistry could do to help the farmer to use his land to better advantage. This could be dealt with by frequent contributions to the agricultural press, by lectures before farm audiences, and especially by his contact with the State Agricultural Societies. Ultimately it led to the writing of "How Crops Grow." There remained the problems involved in direct chemical service.

The Fertilizer Problem

In his lecture at Albany in 1856, Johnson set forth in detail what he believed the scientific method could do for agriculture. As an illustration he referred to the chemical analysis of soils, which many had claimed could distinguish between fertile and barren soils and could

show what amendments and treatments could convert the one into the other. He said,

> Every chemist knows that in the finest kinds of analysis it is impossible to estimate with certainty quantities so small as the hundredths of one per cent. He further knows that in soil-analysis tenths of per cents lie at the limits of accuracy, i.e. it is impossible to determine by aid of analysis between two specimens of the same soil, one of which has been mixed with one-thousandth and the other with two-thousandths of its weight of plaster or guano.
>
> Now, it is not wide from the truth to estimate the quantity of soil in an acre taken to the depth of six or seven inches at 2,000,000 pounds; one-thousandth of this is a ton. A ton of guano applied to an acre would not be estimated by the analyst, and yet we know that three or four hundred pounds is a large application!

He went on to show, however, that chemical analysis of the fertilizers the farmer was offered would indeed furnish information of importance. Furthermore,

> If the manufacturer knew that every month or so, a new analysis of his manure would be published on behalf of the farmer . . . he would find himself compelled to be not only honest, but careful in his business.

The primary and immediate chemical problem was thus the composition of the numerous materials that were being sold to the farmers for use as fertilizer. Many fraudulent preparations were on the market, and there was much uneasiness as a result.

Johnson was well aware that few readers of analytical reports would be able to interpret the meaning of his data for water, organic matter, ammonia, and soluble and insoluble phosphoric acid, together with occasional figures for lime, magnesia, potash, soda, oxide of iron, sulfate, and chloride. Everyone, however, could understand data expressed in terms of dollars and cents. He accordingly adopted a practice he had noted both in England and Germany of putting an estimated valuation on a fertilizer material.

From the market price of fertilizers of known agricultural efficiency, and their analytical composition as determined by himself, he estimated: insoluble phosphoric acid at 4½ cents a pound, soluble phosphoric acid at 12½ cents a pound, actual and some forms of potential ammonia at 14 cents a pound, and potash at 4 cents a pound. He then calculated for each fertilizer a valuation per ton which he compared with the price asked by the manufacturer or dealer. This valuation represented what he thought the farmer could properly afford to pay.

How this worked out may perhaps be best illustrated by the somewhat notorious case of the Mapes superphosphate. In 1852, while still a student at Yale, Johnson had analyzed a specimen of this material obtained in the local market and which he later stated was the "best

that has ever come under my examination." It contained about 10 per cent each of soluble and insoluble phosphoric acid (calculated as P_2O_5) and 2.8 per cent of ammonia. It was valued at $44 per ton and sold for $50. A specimen analyzed in 1856 had only about one-half of this value, and three specimens analyzed in 1857 had only about one-third. He stated in his 1858 Report to the Connecticut Agricultural Society "It is clear that this is a brand not to be depended upon and . . . is hardly worth a long transportation."

The Nitrogen Problem

That a source of nitrogen is essential for the growth of plants was well known to the early investigators such as Liebig in Germany, Mulder in Holland, and Boussingault and Chaptal in France, and of course the benefit to be obtained from the use of animal manure was recognized by the ancients. A century ago the problem could be put in the form of the questions, Where does the nitrogen come from? and, In what form is it used by the plant? Liebig in 1840 had maintained that nitrogen is acquired by the plant from the atmosphere, largely in the form of ammonia brought down by the rain. Boussingault in 1856 demonstrated, in a brilliant experiment, that nitrogen in the form of nitrate mixed with an otherwise carbon and nitrogen-free soil, obtained by mixing brick dust and sand and igniting it, sufficed to bring a sunflower plant to maturity provided that the ash obtained from clover plants was also added to the artificial soil. That he had incidentally sterilized the soil by the ignition was a point the significance of which was overlooked for many years. Lawes and Gilbert in England answered Liebig's view of the situation by collecting all atmospheric precipitation for an entire year in a rain gauge with an area of 0.001 of an acre (4.6 sq. yards). Analyses of the water for ammonia and for nitric acid each month showed that the total nitrogen collected amounted to only from 6.5 to 8.3 pounds per acre, and a similar experiment by Bretschneider in Germany gave only 11 pounds. A crop of wheat would take some 45 pounds of nitrogen from an acre, and one of clover perhaps three times this much. Clearly, therefore, Liebig's view of the situation was inadequate, and the question of the form of nitrogen used by the plant remained uncertain.

In 1867, Johnson gave a lecture before the Connecticut State Board of Agriculture "On the Sources and Supply of Nitrogen to Crops." He began with a discussion of the part that nitrogen plays in the nutrition of the animal, in particular of man.

> Those ingredients of the food which the animal converts into its working tissues contain invariably about 15 per cent of nitrogen. This element exists to the same amount in muscles, in tendons, in the nerves and other essential parts of the animal body that are of organic origin. More than this, the

> animal itself has no power to construct a solitary atom of the material out of which it makes its own muscles and tendons. It finds this material ready made in plants, which are primarily the food of all animals.

This is a clear statement of the doctrine advocated by Liebig, and goes back to the origin of the word "protein," a term originally suggested by Berzelius in a letter to G. J. Mulder in 1838, and derived from a Greek word meaning to take the first place. Berzelius held that the albuminous substances which form the bulk of animal tissue are derived exclusively from plants eaten by the animal. That such substances are of the first importance in nutrition led him to suggest the term "protein" as a convenient name for them. His idea was immediately taken up by Mulder, and through him by Liebig. Liebig, however, never mentioned the source of the idea in his own publications.

In 1867 Johnson was entirely clear that plants obtain their carbon from the air, although there was much contemporary opinion that the so-called humus of the soil was also an important source. But Boussingault in France had shown that plants cannot make use of the free nitrogen of the air. Thus the nitrogen must come from the soil, and salts of both ammonia and nitric acid were widely and successfully used as fertilizers. In fact, until only a few years before this lecture it had been supposed that ammonia in the soil is, as Johnson put it,

> the natural and proper food of plants as regards a supply of nitrogen. It had indeed long been known that nitrates aid in the growth of plants, but Liebig taught that it was probable that nitrates are converted into ammonia in the soil, and, in any case, the main source of nitrogen for vegetation was ammonia.

However, Johnson was now convinced that nitrates "are the chief dependence of the plant for nitrogenous food." Analysis showed that the soil in fact contains only traces of ammonia, but the formation of nitrates in the soil was a well-known process. The manufacture of "saltpetre" from extracts of rich soil was an important industry. Boussingault had studied the rate and extent of nitrification in a fertile garden soil kept moist and under shelter, and had found that nitric acid increased eight-fold during August and early September finally reaching a level two to three times greater than would be regarded as an adequate dressing of nitrate fertilizer. Nevertheless, pot experiments with small amounts of this same soil gave plants that weighed only about four times as much as the seeds that were planted in them in spite of the fact that there was at least twice as much total nitrogen present in the soil in the pot as had been found sufficient to grow a sunflower plant to maturity when the nitrogen was supplied in the form of nitrate to a pot containing ignited brick dust and sand. Clearly, therefore, most of the nitrogen in a soil is present in a form that is not available to the plant, and Boussingault's data indicated that less than one per cent of

the soil nitrogen could be used by the plant. As Johnson put it, "it must . . . [be] for the most part unassimilable and inert, as is the free nitrogen of the atmosphere."

It is a curious fact that no one appeared to wonder very much about the exact nature of this inert nitrogen in the soil. Johnson thought that it exists in the same form as that in peat or in bituminous coal, that is, essentially derived from residues of stubble, plant roots and so forth. That a substantial part of it could, under suitable conditions, be transformed into nitrate was obvious, but the means whereby this reaction was promoted were entirely unknown. The term fermentation was applied, but in 1867 this word did not imply the activity of living microorganisms. It merely meant that the reaction was analogous to that whereby the nitrogenous material of a manure pile was gradually transformed into ammonia, a fact familiar to every farmer.

Johnson's conclusions were that there must be present in the soil a source of nitrogen, that since nitric acid is a compound of nitrogen and oxygen, there must be free oxygen available, and that there must be moisture present at a suitable temperature. Further, nitrification proceeds only in the presence of decaying, that is oxidizing organic matter. His explanation was founded upon observations of Schönbein at Basle in Switzerland who had discovered that oxygen in the air flowing over moist phosphorus is in part converted to ozone. If such a stream of air is then passed through potassium hydroxide, Schönbein claimed that a significant quantity of potassium nitrate is produced. This was supposed to occur by the direct oxidation of nitrogen in the air by the ozone. Johnson stated,

> It has been supposed that other oxidations [i.e. other than the oxidation of phosphorus] are attended with the development of ozone, and it is highly probable that when organic . . . matters decay or oxidize in the soil, ozone is generated which is not recognizable to the chemist, because it expends itself in the conversion of nitrogen into nitric acid. . . . It is in this way that the *free nitrogen of the air* becomes at once part of a compound adapted to nourish plants, and an ingredient of the soil.*

Johnson went on to describe pot experiments of his own which showed that the addition of lime to a soil consisting of peat and the ashes of grass promotes the growth of popcorn to nearly the same extent as the addition of Peruvian guano, and drew the conclusion that,

* This matter of supposed ozone in the atmosphere was taken very seriously at the time and there are even remnants of it today in common speech and in newspaper writing. It has recently become an important agricultural problem. A letter to Johnson from James T. Gardner of the U.S. Geological Expedition in California written from San Francisco on April 1, 1872 says, "I want this summer to make some investigation into the cause of that remarkable haze which prevails over the whole Cordilleras from July till September. It may possibly be connected with the relative amounts of ozone or autozone in the air. Are there any simple tests . . . that we could apply constantly in the field?"

> The action of lime, carbonate of lime or other alkaline fertilizer is, accordingly, to convert inert nitrogen into ammonia. Thus ammonia is either directly absorbed by vegetation or oxidized into nitrates and appropriated by plants in that form. . . . For a number of years it has been regarded as probable that ozone is generated in the act of decomposition which takes place in green foliage under the solar influence, and that the oxygen restored to the air by the decomposition of carbonic acid in the plant, contains an admixture of ozone. . . . It is plain that those crops which produce the largest mass of foliage develop the most ozone during their growth. By the action of this ozone the nitrogen that bathes the leaves is converted into nitric acid which in its turn is absorbed by the plant. The foliage of clover, cut green, and of root crops, maintains its activity until the time the crop is gathered; the supply of nitrates thus keeps pace with the wants of the plant. In case of grain crops, the functions of the foliage decline as the seed begins to develop and the plant's means of providing itself with assimilable nitrogen fail, although the need for it still exists. Furthermore, the clover cut for hay leaves behind much more roots and stubble per acre than grain crops, and the clover stubble is twice as rich in nitrogen as the stubble of ripened grain. . . . Whatever may be the value of our explanations the fact is not to be denied that the soil is enriched in nitrogen by the culture of large leaved plants. . . . On the other hand, the field is impoverished in nitrogen when grain crops are raised upon it.

At the time that Johnson was preparing the manuscript of *How Crops Grow* he was in possession of a great many undoubted facts, and in reading his writings it is sometimes amazing to see how close he came to the truth. But in the absence of any knowledge or understanding of the action of bacteria—for Pasteur's work was only slowly accepted by his contemporaries—there was no guiding principle or theory which could lead to a correct interpretation.

Change in point of view came rapidly in the next decade. A letter written by Johnson to Storer in April, 1875, contains the following sentences. "Before Carius published, I had satisfied myself that pure H_2O and common air yield no NH_4NO_2 at temperatures from 20° to 100° Centigrade.* I never did believe half of Schönbein's facts, and now that Carius finds that ozone don't oxidize nitrogen, I wish old Schönbein were alive again to hear it."

As early as 1858 Boussingault had begun to suspect that there are microorganisms in the soil which are responsible for the transformation of the "inert" nitrogen into ammonia or nitrate thus becoming available to the plant. But the breakthrough did not come until 1877 when Schloessing and Muntz, in experiments designed to purify Paris sewage, filtered the sewage through long columns of either soil or sand. After a few days, nitrate began to appear in the effluent, ammonia disap-

* This sentence is misquoted on page 176 in Mrs. Osborne's "From the Letter Files of S. W. Johnson"; the fundamentally important word "no" was unfortunately omitted.

peared, and they made the shrewd observation that the reaction could be stopped by passing a little chloroform through the column. However, the reaction could be started again if a pinch of garden soil was added. Their work was definitely based upon that of Pasteur, and their conclusion was that nitrification is brought about by "living ferments."

There are several reasons why it took so long to solve the nitrogen problem. As Pasteur found, there was widespread unwillingness to accept explanations based upon invisible microorganisms, and it took years of research before techniques for the isolation and culture of the soil organisms were developed. But to a chemist a major reason is the length of time and the plain hard labor involved in the determination of nitrogen by the analytical methods then available. The Dumas and the Will and Varentrap method, which largely superseded it required heating the sample in a long glass tube charged with reagents in a furnace. Both methods were slow, laborious and intolerably hot especially in summer, and a skilled chemist with two furnaces could at best do only about four in a day. They were accurate in skilled hands, but they could be used only in a well equipped laboratory by highly trained persons.

The Kjeldahl method was first described in 1883, and Johnson picked it up at once and had it carefully studied by his assistants. In the Station Report for 1889, there is a description and picture of a digestion stand and distillation rack for the Kjeldahl method together with devices for measuring the reagents which is obviously the forerunner of the equipment found today in every analytical laboratory.

This equipment is Johnson's greatest personal contribution to analytical chemistry. Compared with the clumsy devices then in use in European laboratories, it is a work of genius, although, to be sure, Johnson had simply put together in convenient form various parts described in the current analytical literature and used for other purposes. But the amount of labor and discomfort that has, in the aggregate, been saved by this ingenious device is quite beyond estimation, to say nothing of the saving of time of the operator. The introduction of the Kjeldahl method was literally a release of the analytical chemist from bondage.

It was the Kjeldahl method in the hands of Wilfarth, who was the discoverer of the use of mercury as catalyst for the digestion, that enabled Hellriegel and Wilfarth so quickly to solve the problem of nitrification by legume plant root nodules, and it was this method which so greatly accelerated the solution of the nitrogen problem, once the share taken by microorganisms had been appreciated.

How Crops Grow

We here at the Experiment Station are apt to think of Johnson only as the Director who, from 1877 until he retired in 1900, supervised the work of the Station, wrote the Annual Reports, kept up with the literature, and at every opportunity broadened the service which the Station

could render to the agricultural public. He was this, to be sure; but he was also a professor in the Sheffield Scientific School at Yale, meeting daily classes in agricultural chemistry and supervising the research of a long succession of graduate students. Such later distinguished agricultural scientists as Jenkins, Atwater, Armsby, Winton, and Chittenden were trained by him, and his influence rapidly spread far beyond New Haven. Perhaps the most important factor in all this was the little textbook he prepared and published in 1868. Its full title was "*How Crops Grow*, a Treatise on the Chemical Composition, Structure, and Life of the Plant, for All Students of Agriculture with numerous Illustrations and Tables of Analysis." The Preface states that it had been his duty for the previous twelve years "to pronounce a course of lectures annually upon Agricultural Chemistry and Physiology to a class in the Scientific School of Yale College. This volume is a result of studies undertaken in preparing these lectures." He goes on to say, "It has been sought to present the subject inductively, to collate and compare, as far as possible, *all* the facts, and so to describe and discuss the methods of investigation that . . . the student may be able to judge himself of their validity and importance." And further, "the writer has kept his eye steadily fixed upon the practical aspects of the subject."

This was the first volume of what Johnson hoped would be a series of four books. The second, "*How Crops Feed*," which Storer a few years later wrote him was even better, appeared in 1870, but the third and fourth volumes, on Cultivation, or the Improvement of Soil and the Crop by Tillage and Manures, and on Stock Feeding and Dairy Produce were never written, in spite of frequent urging in letters from his many friends.

Johnson was physically a frail man. He suffered continually from what today would probably be diagnosed as a stomach ulcer, and he was also subject to frequent devastating headaches the only cure for which was a period in bed. He was greatly overworked, and in 1875 his health was so broken that he had to spend several months in France and Italy in search of relief. This, together with the increase in his responsibilities, when appointed Director of the new Experiment Station in New Haven in 1877, is perhaps sufficient explanation of why the series of books was never completed.

What, then, does *How Crops Grow* contain that made it an immediate success, and which led to its prompt translation into several other languages? The book is divided into three main parts, Chemical Composition of the Plant, The Structure of the Plant and Offices of its Organs, and Life of the Plant. The first part has chapters on the volatile part of plants (organic solids), the ash of plants and the composition in successive stages of growth. After brief treatments of the major elements there is a discussion of cellulose, the pectoses, the vegetable acids, fats and oils and the albuminoids or proteins.

The section on the ash of plants lists the non-metals and metals commonly found in plant ashes which Johnson states are twelve in number,

although he mentions ten or more other elements that are occasionally found in plant ash. The variability of the ash composition of a given species is described, and such questions as what is the normal composition of the ash of a plant are discussed. There is a long section on water culture of plants as carried out in Europe by Wolff, Knop and others, and he also takes up such questions as, Is soda essential for agricultural plants? Can soda replace potash? Are chlorine or silica essential?

The second division of the book begins with a description of the plant cell, its structure and contents, and the facts of cell division as known in 1868, and then goes on to describe the root, stem, leaves, the flowering parts and the fruit. The third division describes the germination of the seed, the sap of the plant, its composition and its motion, and the phenomena associated with membranes. The book ends with an appendix of some seventeen pages of tables showing the composition of the ash of many hundreds of samples of crops, fruits, leaves, etc., taken from the publications by Wolff in Germany as the average of "all trustworthy analyses published up to 1865."

How Crops Grow is still an interesting book. It is thoroughly scholarly; Johnson quotes his authorities, often giving the journal sources. Indeed one of his correspondents later complained that the authorities should be given only in footnotes; the book would then be "less formidable."* The style is simple but formal, the words are carefully chosen, and each paragraph is clear, condensed and specific. It is up to date, for Johnson had already shifted over from equivalent weights for the elements (oxygen 8, carbon 6) to the then new atomic weights (oxygen 16, carbon 12), although many distinguished German scientists such as Bunsen were still publishing in the old style. Finally, it was written in a language that could be understood by any intelligent reader, and, wherever possible, Johnson illustrated his statements by specific references to an agricultural crop, or to some common or even homely experience. For example, in discussing the movements of fluids in tissues, he wrote, "If, however, oiled leather be immersed in water, the oil is gradually and perfectly displaced, as the farmer well knows from his experience with greased boots." Moreover, he was thorough. He had even spotted Heyne's observation published in 1815 that in crassulacean plants (p. 197), "acids are formed during the night which disappear during the day," a matter that began to attract attention only some years after the book was published and which is still a subject for research.

Perhaps the most significant fact about this book is Johnson's choice of a title. It was not "Plants" but "Crops." He was concerned with the farmer. And it was how the crops grew, not how to grow crops. He was concerned with nature, with the mechanisms which the living plant had

* Letter from W. S. Clark of the Massachusetts Agricultural College at Amherst, December 18, 1872. Clark was later sent to Japan where he established the first agricultural school in that country, and where his memory is still revered. There is a statue of him in Hokkaido.

developed to propagate itself and with how man can make use of these mechanisms.

The book attracted immediate attention. G. C. Caldwell wrote from Ithaca, "I have for some time been intending to write to you and tell you how much I am pleased with your work. I use it as a text book in my classes, and it is just the thing that I want to put into the hands of my students." J. B. Lawes at Rothamsted wrote on February 3, 1870, ". . . I received a copy of your book 'How Crops Grow' . . . I hasten to thank you for it, and I have so far gone over its contents as to satisfy me that it is by far the best summary of information in this subject which has yet been published." Hermann von Liebig wrote on November 29, 1869, "Mein Vater gab mir Ihr Werk und wir stimmen darin überein dass es ein sehr gutes Handbuch für Landwirthe ist, und sich auch in Deutschland viele Freunde erwerben würde." He goes on to say that he plans to translate it into German. At the Convention of the Friends of Agricultural Education held in Chicago in August 1871, W. C. Flagg of the Illinois Industrial University, in the course of a debate upon the relative importance of the "practical" versus the "theoretical" approach to agricultural problems said, ". . . Prof. Johnson, in his 'How Crops Grow,' and 'How Crops Feed,' is doing more to advance agricultural interests than any man in the United States. . . . I think there has been nothing more practical or more valuable for getting our general agricultural practice upon a scientific basis, than those works by Prof. Johnson."

How Crops Grow became the standard text in agricultural chemistry in schools and colleges throughout the world. There were a number of reprintings and translations, and Johnson prepared a second edition in 1890. The section on proteins in this edition is by far the best account of the subject in any textbook of the period, and was so for many years thereafter.

The Influence of Johnson on American Agriculture

What exactly did Samuel W. Johnson do that made him the acknowledged leader in agricultural science in America a century ago? One must first realize that he was brought up on a farm by a father who was an able and successful farmer, and who regarded farming as a profession. It took his son several years to persuade him that there was a future for a young man who proposed to go into science with the ambition to improve the conditions of agriculture in this country, but when his father was finally convinced he supported the boy to the extent of his ability. The teachers at the Lowville Academy had given young Johnson a sound foundation in English, with sufficient Latin and Greek to serve him well in later years, and his science teacher had aroused an enthusiasm in him for chemistry, physics, and botany that shaped his entire life. But perhaps the most important thing that he acquired at Lowville was an interest in books, and for the rest of his life he spent every penny he could afford, and often more, in buying books. There

are still in the Station library many of the books he acquired during his student period at Yale and while teaching at Flushing and Albany, and his purchases while a student in Germany, textbooks, reference books, and whole files of scientific journals form the foundation of our library collection to this day. Not only did he buy these books, but he read them, and he read the journals and reports. Even before he went to Germany he must have acquired considerable facility with German and French, and he and his friend Evan Pugh must have haunted the bookshops in Leipzig during the year they spent with Erdmann. Both came home with extensive libraries, and throughout his life at Yale, a copy of every important text or monograph found its way into his collection on publication.

Not only did he acquire knowledge from his reading, but he shared it. At first, of course, owing to the economic pressure he was subjected to, but also with the clear motive to give the farm public the benefit of his reading, every few weeks he wrote an article for the *Country Gentleman* or the *Homestead*. There were seventeen of these in 1856, thirteen in 1857, and subsequently one or more each year for many years. These articles were addressed to the practical farmer. The language was clear and simple; he did not say "Do this" or "Don't do that," but described the results of some agricultural practice which from personal observation or from his reading had been found to be beneficial. The articles attracted the widest attention.

Johnson had brought home the conviction that institutions resembling the *Versuchstationen* then being established in Germany should be set up in America, and he was emphatic on this point as early as the public lecture he gave in Albany in 1857. His position as chemist to the Connecticut State Agricultural Society gave him an opportunity during the next three years to demonstrate what a chemist could do for agriculture. His three annual reports contained analyses of a number of the commercial fertilizers then on the Connecticut market. The third report contains the statement about Mapes' superphosphates already mentioned, and showed quite another aspect of the service a chemist could render. The outbreak of the Civil War in 1861, however, led to the demise of the Agricultural Society, and it was not until 1865 that the Connecticut State Board of Agriculture was set up and Johnson became their official chemist, a position he retained until 1898. Meanwhile, his influence on American agricultural science grew.

Norton, his predecessor at Yale, had encountered firm resistance to his efforts to persuade the farmers of the state that education of their sons in agricultural science would lead to great benefit. Most farmers had little interest in what they called "book farming," and Norton's plans about 1850 for the establishment of schools to provide instruction at several levels, the highest being a State Agricultural College for the training of teachers, received little encouragement either in Connecticut or New York. Nevertheless, the little school at Yale was a reality, and Johnson's enthusiasm, on his return from Germany, and his immediate

and valuable demonstration of scientific services for the farmer obtained recognition at all levels. Efforts were being made to establish agricultural colleges in a number of states. In Pennsylvania, a charter was granted by the legislature in 1855, and a committee, headed by Dr. A. L. Elwyn, was appointed to select a principal. Elwyn wrote to Yale, possibly to Professor Silliman, but the letter was answered by Johnson who had just returned from Germany. It chanced that his close friend, Evan Pugh, then studying with Wöhler in Göttingen, had just written asking if he knew of any agricultural schools, "likely to go into operation" within the next year or so, and Johnson accordingly suggested Pugh's name to the Pennsylvania group. Elwyn's prompt offer to Pugh gave rise to a number of letters between Pugh and Johnson in which Pugh set forth his ideas for education in scientific agriculture, ideas that he later put into operation at what ultimately became the Pennsylvania State University, but which was at first known as the Pennsylvania Farmers' High School, a name carefully chosen to avoid any connotation of the term "college" that the local farm public might regard with suspicion. Pugh returned to America in 1859, after two further years of study with Lawes and Gilbert at Rothamsted and, during the next five years of his tragically short life, transformed the Farmers' High School into the Pennsylvania State College of Agriculture, a successful land-grant college which, although with various vicissitudes at first, ultimately became a leader in the field. Pugh's career both with respect to brevity and to success bears the closest analogy to that of John Pitkin Norton at Yale.

Johnson's first move in providing for advanced education in agriculture in America thus bore extraordinary fruit. It was the first of many. Through his influence George C. Caldwell, Pugh's successor at Pennsylvania, eventually got the appointment at Cornell in Ithaca. His student Henry P. Armsby, Yale Ph.D. in 1879, chemist at The Connecticut Agricultural Experiment Station 1877 to 1881 became professor of agricultural chemistry at Wisconsin in 1883, and Director of the Pennsylvania Experiment Station when it was established in 1887. Another student, Wilbur O. Atwater, was appointed chemist at Knoxville, Tennessee in 1869, and transferred to Orono, Maine in 1873 as a result of letters written by Johnson, but moved to Wesleyan College in Middletown, Connecticut in the fall of that year. Edward H. Jenkins, after spending two years in Germany, returned to a position with Atwater during the second year of his directorship of the Connecticut Station at Middletown, and was then appointed chemist by Johnson when the Station was set up in New Haven in 1877. He succeeded Johnson as Director in 1900. Among later students were such men as Andrew L. Winton, Ph.D. 1884, who was a chemist at the Experiment Station from 1884 to 1907 and later the head of the Food and Drug Laboratory of the U.S.D.A. in Chicago and a distinguished authority on nutrition. And no account of Johnson's eminence as a teacher should omit reference to Russell H. Chittenden who established the first laboratory of physiological chemistry in Amer-

ica in the rooms left vacant at Sheffield Hall when the Experiment Station moved from there in 1882 to its present location, and to his son-in-law Thomas B. Osborne who joined the Station staff in 1886 as chemist.

Recognition by the scientific fraternity of Johnson's eminence came in 1866 when, at age 36, he was elected to the National Academy of Science, and there were a number of attempts to lure him away from Yale. Perhaps the most interesting of these was in a letter from Joseph Henry, the Secretary of the Smithsonian Institution written in July 1871. Henry proposed that Johnson should, "take charge of the operations of the chemical branch of the Agricultural Department without severing your connection with the scientific school of Yale College. . . . It appears certain that you can have the appointment if you will accept it without restriction. While I would not advise you to do this I still think you might be induced to take charge of the operations and, with the assistance of your pupils, do work which would redound to the advantage of agriculture and the reputation of the Agricultural Department." In September, Commissioner Watts made a direct offer with the statement "I will appoint no one who has not the recommendation of known men of science. If you will not accept the place can you name to me any one whose appointment would be approved by the scientific men of the country." Johnson's reply is not available, but during Watts' absence later in the month, the acting commissioner thanked him for his suggestion and expressed his "appreciation for the kind feeling which you evince."

The Beginning of the Experiment Station

In the time remaining, I can give only a brief account of the steps that were taken, beginning at the meeting of the State Board of Agriculture in late December, 1873, to realize Johnson's ambition to establish an experiment station in Connecticut. The facts are as follows. A committee of farmers were to prepare a bill and submit it to the Legislature, and attend hearings before the Legislative Committee on Agriculture. At the meeting of the Board in December, 1874, they reported that, in spite of having obtained a petition signed by more than seven hundred farmers, the Committee on Agriculture had decided to lay the bill over for the following year. Opposition had developed in the Committee from several of the farmers who were members, although members from the business world were in favor.

This failure was taken very seriously by many members of the State Board and led to a sharp debate. One of the leaders of this debate was Mr. Orange Judd, a Trustee of Wesleyan University and a wealthy publisher and classmate of Johnson when a student at Yale. Impatient with the action of the Legislative Committee and apparently on impulse, he rose to propose that the group present could easily raise a thousand dollars by personal subscription to start an experiment station "within

ten days." He offered the use of a large laboratory in the new science building at Wesleyan, together with the services of the newly appointed young professor of chemistry, W. O. Atwater, who could be relieved of some of his teaching responsibilities so as to supervise the work.

The outcome of the debate was that the committee of the State Board was instructed to continue its efforts to arouse the interest of the farmers of the state, and to appear again before the Legislature to urge the passage of the bill. However, the idea of raising a public subscription in support of a station was not approved. The committee's efforts were successful, although the legislature cut down the appropriation from the desired $8000 to $2400, and Mr. Judd's personal offer of an additional $1000 was accepted. The bill was passed on July 20, 1875. The appropriation was made for a period of two years, and was to be paid to the Trustees of Wesleyan University who were to supervise its expenditure.

In spite of his embarrassment at being preferred to the position of Director instead of his honored teacher Johnson, Atwater accepted the position, and got the station under way in October. A meeting of farmers and of manufacturers of fertilizers was organized a few weeks later, and nearly forty of the prominent dealers signed agreements to submit their stocks to analysis by the chemists of the station. A fee was collected for these analyses. Farmers were also urged to submit samples of fertilizer they had bought, and it was strongly suggested that they should buy only from dealers who had signed the agreement with the station.

In the late summer of 1876, Johnson's former student Dr. E. H. Jenkins and Mr. Georg Warnicke, both of whom had been studying the methods of seed analysis with Professor Nobbe in Germany, were employed as chemists at the station in Middletown, and an amusing letter from Jenkins to Johnson, dated in March, 1877, reported that "The Experiment Station floats and the crew are all pumping and bailing, having a heavy cargo of fertilizers and seeds under examination."

However, it had become clear to the State Board of Agriculture that the experiment station entirely under the control of the Trustees of Wesleyan University in Middletown, although remarkably successful, was only a temporary and by no means a satisfactory solution of the problem. It was itself an experiment. A bill was accordingly prepared and submitted to the Legislature early in 1877 and was approved on March 21. It appropriated $5000 and set up a Board of Control of eight members representing the State Agricultural Society, the Board of Agriculture, Wesleyan University, the Sheffield Scientific School and the Governor of the State. The Governor and the Director of the Station were to be *ex officio* members. Professor Samuel Johnson was empowered to call the first meeting of the Board as soon as practicable, and this Board was to have the general management of the station.

This Board met in April 1877, appointed Johnson as Director, and accepted the invitation of Professor Brewer to occupy rooms in Sheffield Hall at Yale. Johnson at once invited Jenkins and his former student H. P.

Armsby to join the staff as chemists, and the first report of their work, a circular written by hand with the Edison electric pen and circulated to the newspapers of the State giving the composition of a fraudulent fertilizer priced at $32 a ton but worth $1.03, is dated August 18, 1877.

Johnson's ambition, the establishment of an experiment station, organized on the proper lines, supported by an appropriation from the State and the fees charged dealers for analyses, and with its own Board of Control essentially free from any political influence, was thus finally realized. The prompt success of the Connecticut station, and the rapid spread of the movement to other states, where the stations are usually associated with the state agricultural colleges, shows the influence of "the company of capable investigators" that Johnson hoped would rise up from the study of his textbook.

In the America of 1850 about nine workers out of ten were employed on farms or dealt with agricultural products. Today, the ratio is reversed—about one worker on the farm for fourteen in other industries. The change, with its accompaniment of enormously increased wealth, is a direct result of the application of science to agriculture, and Johnson was one of a very small group of men a century ago who took the steps which led to this transformation. Exactly who was the first is unimportant; one of them was certainly John Pitkin Norton at Yale in the late 1840's. In Europe, the leaders were Liebig in Germany, Johnston in Scotland, Boussingault in France, and Lawes and Gilbert in England. By the 1850's, there were many others in the European universities and *Versuchstationen.* Johnson in this country served as the interpreter of their work, and by his example and service as a professional chemist, and through his influence as a teacher, a rapidly increasing number of practical farmers learned how best to use fertilizer, how best to feed their stock, and how to maintain their soil.

Progress was indeed slow at first. It was the farmers in the Connecticut Legislature who resisted the experiment station idea in 1874, and their support given the following year was grudging to say the least, but once land-grant colleges and experiment stations spread across the country during the next fifteen years, the pace accelerated. Fundamental research in bacteriology, genetics, nutrition, and many other branches of biology, to say nothing of the progress in chemistry, have led directly to the spectacular transformation of which we today enjoy the benefit. The release of the farm worker to go into industry, the accumulation of wealth which resulted, the development of new crops, and of means for the control of plant diseases and of harmful insects, and above all the invention of new machines and sources of power for the farm may all be traced back to the influence of that small group of men who, a century ago, had the vision to see what science could do for agriculture. Of these men, Samuel W. Johnson was the acknowledged leader.

September 17, 1968

How Crops Develop: the Past, the Present, the Future

F. C. Steward

It is a pleasure for me to be here for several reasons. I have referred, as others have, to this Station as one of the best of the agricultural experiment stations of the United States, and that this is not unassociated with the fact that it is also one of the smallest. I am a believer in the fact, being a little fellow, that it is very difficult to have both quality and size. There are certain penalties in being big; the bigger the institution, the more and the greater the problems it encounters.

I am also pleased to be here because I have had contact with some honored names associated with this Station. I am proud to count Dr. Vickery as one of my scientific friends; and I also knew, in England, a great friend of his, also associated with Yale in earlier days, namely Professor Chibnal, and I knew your Director as a graduate student at Cornell.

First we should look back a hundred years. What sort of setting was it in 1868? I will shortly say a little about the origins of plant physiology, but the 19th century was, of course, one of the great centuries in which a tremendous wave of scientific development and philosophy occurred. It opened auspiciously for plant physiologists in 1804 with De Saussure's book entitled *Recherches Chimiques sur la Vegetation,* and what a neglected volume that is. I should not "tell tales out of school," but when I went to Cornell in 1950 I was about to lecture in March of the following year on a variety of topics, and I thought I should have this old book by De Saussure for easy reference. So I withdrew it from the library—a beautifully bound copy—which, somewhat to my surprise, I could obtain without restriction. But, in the front, there was a slip which indicated that it was last withdrawn in 1937, and it was then 1951! Then I turned to the back, and there was another withdrawal slip, yellow with age, on which there was a single entry in 1901! That treasure of plant physiology had only been drawn from the Cornell library, according to the record, about three or four times. Well, I read my class a sermon about this, but I'll tell you the sequel. Since nobody else seems interested in this book, it resides on my desk more or less in perpetuity—I sign it out every year! Because if others do not wish to consult it I do; and moreover I like at least to touch the cover and know that at any time I can open it up and establish a sense of contact between myself and De Saussure in 1804.

But you remember what happened. The 19th century encountered the travail of the Napoleonic Wars and their aftermath. The aftermath of war is always good for science, that is the tragedy, and in this case it was good for agricultural science. The economy of Western Europe was prostrate; and so Liebig in Germany, Boussingault in France, Lawes and Gilbert in Britain, and the other figures that we are going to hear about in the United States caught on to the idea of an artificial fertilizer industry and the concept of helping nature along with the business of nourishing plants so that they could the more effectively serve as crop plants. But, in a more academic sense, think of the tremendous things that also happened: the discovery of the nucleus in 1831, the cell doctrine in 1838-39, and later in the century the discovery of mitosis; de Vries and Pfeffer, with their work on plasmolysis and osmotic systems, making measurements which, as botanists, we should remember supplied the data that permitted physical chemists to invent the theory of solution. In fact, I first met the term "isotonic" long before I studied plant physiology, because I learned it in physical chemistry and only realized afterwards that de Vries had used it to describe solutions which, at incipient plasmolysis, produced in cells a condition of "equal tonus," for they all had "equal attraction for water." Then in the great onward march of events there was a really tremendous wave of progress, so that by the end of the 19th century and the beginning of the 20th the foundations of modern plant physiology were laid, and we had a system of physiology and agriculture based on a rational system of chemistry.

But what about that date, 1868? It is interesting to note that Sachs (1875), who wrote the best history of botany, stopped it at 1860; but why? He did this because it was the end of one era and the beginning of another, for it marked the onset of the great wave of application of modern physics and chemistry, of physiology and cell physiology, to the study of plants. And when Reynolds Green who, as an author of a widely used text in Britain (1900), was a later counterpart of your Johnson, came to write the history of the rest of the century he needed a whole volume for the 40 years from 1860 to 1900. Sachs described it all from Aristotle to 1860 in one volume, but in the companion volume, published by the Oxford Press, Reynolds Green (1909) needed a whole volume for that 40 years alone. It was in that explosive setting that your Station was founded, and the sense of drama is recaptured if one recalls the great generalizations and axioms that came out of the 19th century: "all cells from pre-existing cells"; "all nuclei from pre-existing nuclei"; "protoplasm is the physical basis of life"; "the nucleus is the physical basis of inheritance"; and, a little later, these led on to the great aphorisms of today, if you care to trace them. So that's the sort of perspective we get by looking back. And you note that all these aphorisms relate to the transmission of likeness in the continuity of structures. As we shall see, our present preoccupation is with the means by which, within this system, great diversity is achieved.

But I wish to look back even further to the very origins of plant physiology.

We all know about Stephen Hales, who is a great favorite of mine, for in 1727, in the book *Vegetable Staticks* he laid the foundation of plant physiology (at least for the English speaking world). But I suppose everyone also knows about Van Helmont and the experiment with a willow twig, performed in the early 16th century. Again, you see, it is an experiment on growth. This was the problem that captivated people from the outset. Why do things grow, and how and whence do they derive their substance? And if you really want ultimate answers, we still don't know so very much about it. We know much about *how* things grow, but not why. Can one even today say *why* a plant cell actually divides? To grow and divide it must be nourished, to use and absorb nutrients it must grow; but what "winds up" the machinery in the first place is still mysterious. But when did plant physiology really begin? Let me illustrate the interest of historical inquiry by something recently published with one of my former graduate students (Krikorian and Steward, 1968).

It has been said that Nicholas of Cusa anticipated Van Helmont by 150 years. And so Krikorian and I, knowing about Stephen Hales and Van Helmont, consulted the first English translation of the extensive works of Cusa. We did this first in *The Idiota* (the term "idiot" is not here a term of reproach, for it in effect refers to an ordinary man in the street) in the English translation of 1650 and later in the original Latin text, published in the early 1400's. The publication in question (Krikorian and Steward, 1968) reproduced a photograph from the manuscript in the British Museum, which shows that the famous passage of Van Helmont about the willow tree was essentially all in the works of Cusa. And, if one quotes from the translation of 1650, the essential passage reads as follows in the rhetorical style in which the "Idiot" responds to the questions posed by the "Orator."

> "Orator: There is a saying, that no pure element is to be given, how is this prov'd by the Ballance?
>
> Idiot: If a man should put an hundred weight of earth into a great earthen pot, and then should take some Herbs, and Seeds, and weigh them, and then plant or sow them in that pot, and then should let them grow there so long, untill hee had successively by little and little gotten an hundred weight of them, hee would finde the earth but very little diminished, when he came to weigh it againe: by which he might gather, that all the aforesaid herbs, had their weigh from the water. Therefore the waters being ingrossed (or impregnated) in the earth, attracted a terrestreity, and by the opperation of the Sunne, upon the Herb were condensed (or were condensed into an Herb.) If those Herbs bee then burn't to ashes, mayest not thou guesse by the diversity of the weights of all; How much earth though foundest more than the hundred weight, and then conclude that the water brought all that?"

These words almost paraphrase the more familiar ones of Van Helmont; thus Van Helmont in the early 16th century, performing his willow tree experiment, must have had some devious connection with Nicholas of Cusa in the early 15th century. This then was an early experiment on growth, and those now in this Station who are interested in water relations, and I know there are several, are really in lineal scientific descent with Robert Boyle and Van Helmont and Nicholas of Cusa going way back to the early 1400's and they are thus a part of an honorable tradition.

But when, in the early history of natural science we think of Stephen Hales we naturally think about the pressure of vine sap and putting manometers on the arteries of cows in the church yard of Teddington near Kingston, Surrey (near where I used to live when I was in London) to measure their arterial blood pressure and to compare with this the flow of the sap of vines. But Stephen Hales also investigated how plants grow, and he invented some primitive kinds of auxanometers, and these are illustrated in *Vegetable Staticks* (of Statical Essays, Vol. I, 3rd edition 1738, pp. 346, 351). He made a board, put nails in it, stuck the nails through leaves, and then he watched the changing pattern of the holes as the leaves expanded. This anticipated the familiar work of Richards and Kavanagh (1945) on tobacco leaves by nearly two and a half centuries. And there was also a somewhat crude but effective device, comparable to those that are now used to measure the growth of coleoptiles. Stephen Hales, however, used a wooden spike pierced with equidistant nails, and with this he made holes in the expanding internodes of stems and, as they elongated, he noted the distribution of growth in the internodes. There it all is in *Vegetable Staticks* in the early 1700's, and one may re-read the significant words of Hales (1738 loc. cit. Figs. 40, 41 and 42, p. 346). This is the charm of history, for one may still go back to the people who started it all, and the incredible thing is how much they did with so little; by contrast, this makes one feel chastened, since we often do so little with so much.

But now let us return to the 19th century. Another great event was the discovery of the embryo sac and the mechanism of fertilization in an angiosperm and the significance of the endosperm and, of course, the discovery of chromosomes. But as we reach the end of the 19th century the great figure of Haberlandt emerges, for he touched the subject of botany at so many points, and had the insight to anticipate one of the great problems of today about how plants grow. If equational cell division means what it says, if a cell as it divides makes a facsimile of itself, then as the egg divides it ought to transmit to each daughter cell the genome, i.e. all the information to recapitulate the whole plant. There is, however, something very mysterious about the fact that when two cells remain attached (and I will return to this point) "division of labor" enters in, and neither of them achieves wholly and fully all of what each is capable. But Haberlandt had the insight to say, in effect, that 'one of these days we ought to be able to take free

cells and grow whole plants.' But in fact he even said more, for, in 1902, in a somewhat inaccessible journal which many have cited and very few seem to have read, there is an illustration which I have recently copied (c.f. Growth and Organisation in Plants, p. 486). This paper contained very little data; nevertheless Haberlandt showed that cells like those of *Lamium, Tradescantia* hair cells, etc. could separately survive, exhibit streaming, thickening their walls a little, although, by modern standards, they showed virtually nothing of their inherent ability to grow. Even so Haberlandt saw that one of these days we ought to be able to make plants out of such free cells. At least that is what I always thought he said until, not very long ago, I noted that he really said that "out of cells like these we ought to be able to make *artificial embryos.*" And this is the part of my talk that will now bring us down to the present and to an anticipatory look into the future. So now we jump from Haberlandt in 1902 to the 1960's.

In the beaker illustrated (Fig. 4C) are literally hundreds and even thousands of tiny carrot plants all perfect in all essential detail; in fact all could be grown to maturity. But they all went into that culture in one flask as free cells, derived from parenchyma (that is from adult, mature differentiated tissue) so that Haberlandt's dream has in fact come true. Of course, to do this we have performed a number of chemical tricks, but, nevertheless, the philosophical concept is that during all the devious processes of growth and development the information that entered the zygote at fertilization remained intact in all those living cells ready to be exploited by the right kind of exogenous treatments, or the appropriate environmental factors.

Some landmarks along the way should be noted. In my laboratory, the first event occurred when we were trying to do something else. So often the best things in science have happened almost by chance, for they could not be completely pre-planned. In this case, we took storage tissues like those of potato, carrot or Jerusalem artichoke and, for purposes of studying metabolism and salt intake, tried to make them grow again as rapidly as they could, so that we could reversibly place the tissue in the contrasted growing and non-growing states. All the details may be omitted here. However, we essentially thought of what happens to a cell in the embryo sac, for there the zygote is nourished by a fluid, the liquid endosperm, and the stuff that is in liquid endosperm is also in coconut milk (or coconut water as it is sometimes called). But if you put a piece of carrot on an otherwise complete medium, it may merely sit there and not do very much, at least if it is small (2 mgm) and appropriately cut from the carrot root at a distance of 2-3 mm. from the cambium. But if you give an equivalent piece some coconut milk in the basal medium, it may then grow very rapidly (Fig. 1); and I think Johnson (1868), the author of *How Crops Grow* and later (1870) of *How Crops Feed,* would have been as thrilled as I was when I first saw that response. But this growth under these conditions was uncoordinated, proliferated growth, although it occurred at a truly tre-

mendous rate. But we now do this under controlled, aseptic, standardized conditions for purposes of our research, and the methods have been described (Steward, Caplin and Millar, 1952). Hundreds of little (2 mgm) carrot explants, all cut from a single carrot root at a known distance from the cambium, are dispensed, one to three at a time, into tubes with 10 ml of the appropriate medium, and these are put on a slowly revolving wheel; and, as the wheels go round, the tubes expose the tissue, alternately to liquid and to air, and the explants then grow very rapidly. Alternatively many (100) such explants may be grown uniformly in special one liter flasks (Steward and Shantz, 1955; Steward, 1963) in response to the stimuli in the coconut milk, whereas in its absence they remain but little changed. The little carrot explants, on a complete basal medium, receiving everything (salts and trace elements and sugars and vitamins) that should induce the carrot explants to grow rapidly may, nevertheless, only expand sluggishly, with relatively few cell divisions. However, if one supplies the contents of coconut milk, such 2 to 3 mgm of initial carrot explant may grow to 200 to 300 mgms in 20 days! Or 25,000 initial cells increase to between 2½ and 3 million cells; and, in doing so, chloroplasts are formed in the light, and all aspects of cellular organization are created so that protein synthesis and ion uptake take place. This is the experimental system out of which the development from free cells came about. The routine procedure is as follows.

One hundred carrot explants, cut from a single root, are placed aseptically in a liquid medium either with, or without, the coconut milk. The explants exposed in a flask to either the coconut milk, or other combinations of growth factors which now will do as well, grow into nearly spherical, green, balls of tissue; this constitutes marvelous material on which to study the biochemistry of actively growing cells. But as the flasks turn around the little balls of tissue rub against each other and gently push a few cells off into the medium. But, doing it gently, the cells that are pushed off into the medium remain suspended and constitute cells that can grow, in a medium which makes them grow, and in a space which allows them to grow; and so they do grow, but in a very different manner than the cells attached to the tissue piece. Being free, unhampered by organic connection with their neighbors, they express their intrinsic properties, and in fact the free cells may behave like zygotes and develop in an embryological way and grow into plants. In appearance the cells *in situ* in the carrot are very different from the cells that grow free (c.f. Steward 1961), so that one might think they were not even from the same plant. Nevertheless, the free cells can be grown in great quantity by these means if desired.

Therefore, we now have a technique to obtain free cells, we can filter the suspension so that almost all the cells are single and only a very few may be doublets that were already divided in the medium. One can filter such cells and spread them like bacteria on petri plates, and although more slowly than bacteria, they grow into colonies. Thus, one

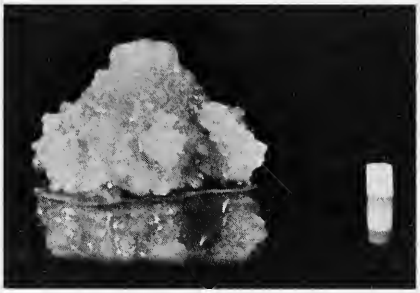


Fig. 1. Original observation on growth induced in carrot phloem explants by coconut milk: right, typical 2.5 mg explant on a basal agar medium after White; left, typical growth of a similar explant on similar medium supplemented with coconut milk (10% by vol.).

can now do many of the standard bacteriological techniques with angiosperm cells. But the dramatic result is that Haberlandt's prophecy has now been fulfilled.

To accomplish this, we first obtain the cells *free* and expose them to a medium in which they can fully exploit their innate ability to grow, so that they re-express all the attributes of the zygote from which they were originally derived. Although this came about in a somewhat fumbling way at first, we soon saw that the divisions of the suspended cells were not random, but orderly. Thus the small groups of cells which developed in the free floating state were organized, and it became apparent that they were, as it were, "trying to behave like embryos" Steward, Mapes and Mears (1958), and their behavior compared favorably [Steward et al. (1961)] with the embryogeny of the cultivated carrot as described by Borthwick, and as here reprinted from Wardlaw (1955) (Fig. 2). In fact, it was soon seen that free cells, placed in the tubes in which we normally grow small explants of carrot tissue into proliferated callus masses, form roots with the greatest of ease, even in the same culture medium. But for some time we did not readily

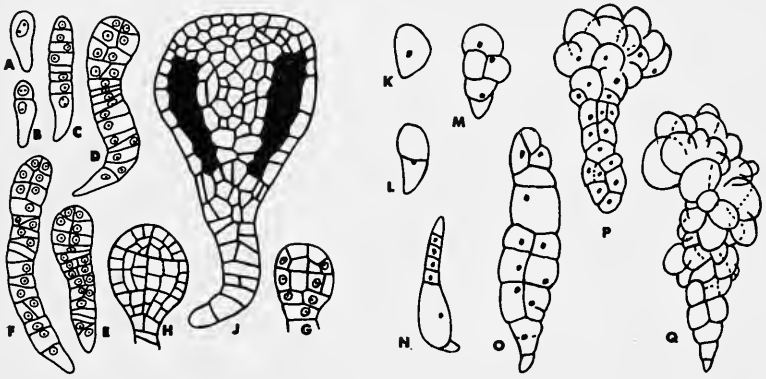


Fig. 2. A-J, normal embryogeny of carrot after Borthwick and reproduced from Wardlaw; K-Q, representative cells and cell clusters grown in liquid suspensions showing various degrees of organization.

obtain shoots, but those who follow the literature of these things will now know that, over ten years ago, we grew free cells of carrots into little cell clumps with initiating roots, which were sowed on agar, and from these proliferated but organized masses one could remove large numbers of embryoids, which were so like zygotic embryos, having two cotyledons an axis and a root tip, that the differences were immaterial. And these embryoids were grown into whole plants, with real storage carrot roots rich in stored carotene, and eventually to flowers. So, 60 years later, Haberlandt's dream had come true.

But even so it is remarkable how faithfully parenchyma cells, if appropriately treated, can rediscover the ability to behave like zygotes. The results show that they may give rise, faithfully, to an embryological development which includes globular embryos, heart-shaped embryos as nicely formed as any ever dissected from an ovule (like that of Shepherd's purse), or a developing seed. But all this development may come about in a flask, in a population of cells which seemingly ought to have forgotten how to be embryos many, many cell generations ago; nevertheless they form torpedo-shaped embryos then cotyledonary embryos, which turn green at their tips, and which initiate secondary thickening as they begin to form a storage root (Fig. 3).

But another development came about, again partly by accident. People said, "ah yes, you've grown carrots, you've made carrot roots, you've grown flowers, but do the flowers have seed in them and will the seed grow?" So we tested this point, although we never had any doubts, but nevertheless this test produced an unexpected dividend. We grew free cells from the carrot root, formed developing embryoids with roots, removed many embryos, and grew them into plants which flowered; we then removed a still immature embryo from a flower on a plant grown originally from a free cell. And when we put this young embryonic plantlet into an agar medium, which also contained coconut milk, it grew into a small plant. But as we did this we also brushed off some cells from the hypocotyl region on to the surface of the medium, and

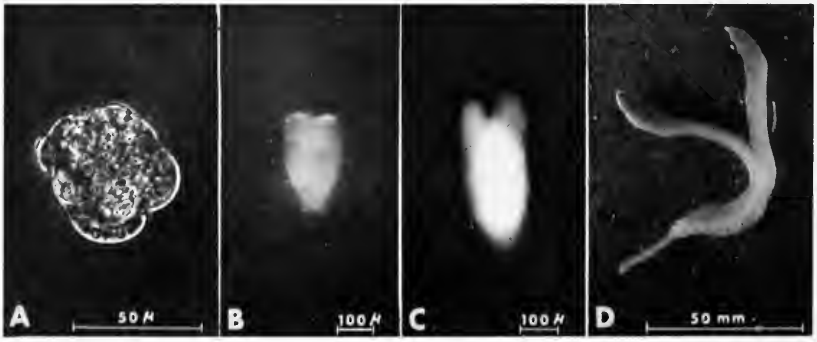


Fig. 3. A-D, representative stages of embryo development from free carrot cells of embryo origin.

from these cells perfect heart-shaped and torpedo embryos appeared on the medium (Steward and Mapes, 1963). This gave us another device, for we could now start cell cultures from immature embryos, and they then go through this embryonic development again with ease. Later, with one of my graduate students (Kent, 1966), we took a flower out of the wild carrot of my garden (there were lots of wild carrots in my garden, I might say), and from this we took one embryo and made it proliferate, and so obtained a copious free-cell suspension which was filtered and spread upon a petri dish, so that an estimated *one hundred thousand* of organized structures appeared on the agar surface (Steward et al., 1964). These cells did not form unorganized colonies for they developed into the globular, heart-shaped, torpedo embryos and then, if transplanted, formed plants with flowers, thus recapitulating the normal processes of growth and development. Therefore, the information that was in the zygote was also in the parenchyma cells all the time, waiting to be evoked by the appropriate external conditions and stimuli. Thus, we can have a complete carrot life cycle without any of the sexual process. Mature cells of the carrot phloem produce callus cultures, then free cells, and embryogenesis leading to embryos and plants with storage carrot roots. Normally the storage root would produce a flowering spike in the next cycle of growth and then multiply by seed, but we need not wait for this, for we may now start the cycle off again from root phloem cells and then repeat it indefinitely (Steward and Mapes, 1963). And every time we do this, so many times that I have lost count of it, it always works the first time, producing a complete carrot life cycle bridged at the free cell stage by isolated parenchyma and without any part of the sexual process.

But can we do this with any other kinds of cells? When free cells are cultured in this way they may resemble each other despite the plant of their origin, or unless they happen to be distinctively pigmented, as with anthocyanin (Blakely and Steward, 1961) even as fertilized eggs look alike. A published figure (Steward, 1966) showed six examples of free cells, five of which were obviously activated cells with conspicuous nuclei, cytoplasmic strands, streaming cytoplasm, even dividing, which were obviously en route "to behave like embryos." But one clone of cells seemed indifferent, and this happened to be from an asparagus culture. And then, by one of those fortunate laboratory accidents, a technician returned the culture, that had been growing slowly for months on a synergistic mixture of coconut milk and naphthaleneacetic acid, to coconut milk and 2,4-D which, when first tried, was ineffective. Now the culture grew rapidly, and the cells when examined were very different, for instead of being empty-looking and seemingly inactive, they now had full contents and were obviously reactivated. Thus it takes more than a single substance to unlock the door of cell division; but a sequence of stimuli may be required. In effect, one also has to do the right things, in the right order. There is no point in trying to do stage 5 until you've accomplished stage 3. And

this is what we now call the sequential effect (Steward, Kent and Mapes, 1966, 1967). Since differentiation is a planned sequence of events in time, the reverse process of converting mature cells to reactivated embryonic ones also requires a planned time sequence of stimuli. By the aid of the sequential effect, some truly remarkable things have been accomplished. A simple plan is used to find the appropriate conditions in any given case; this can be illustrated for *Nicotiana.* First we find the conditions compatible with the maximum cell proliferation and unorganized growth; to do this, many combinations of stimuli are arranged as in a Latin square diagram. Tissue explants are subjected to the treatments in small disposable petri dishes. In the case of *Nicotiana,* one could see that some morphogenesis occurred with little growth under one set of conditions (B + CM) and cell proliferation under another (B + CM + NAA).* Therefore, we develop the crop of cells under one set of conditions (B + CM + NAA), then take them off that medium and put them back on to the one that fosters organization (B + CM). In employing this sequential effect it is often found that a medium containing a combination of coconut milk and napthaleneacetic acid gives us a copious crop of cells; but, to foster the organization, the naphthaleneacetic acid is removed and, if the growth persists on either the medium with the coconut milk, or even on the basal medium, morphogenesis ensues. In this way we grew free cells of tobacco with full green chloroplasts, and from these quickly produced our first tobacco plantlet some years ago. In fact, the tobacco plant grew to flower in a test tube faster than it normally would have done from seed (Steward, Kent and Mapes, 1966).

But to show that all this can now happen on a large scale, cultures of *Sium suave,* the water parsnip, may be cited (Fig. 4); these were obtained after sequences of operations similar to these described. Free cells developed a large crop of plantlets (Fig. 4A-C), as shown by a picture of a petri dish (Fig. 4B) with the contents of but one culture flask containing thousands of plantlets, all perfect in every detail (Fig. 4D), although they arose from free cells and not from fertilized eggs as in seeds (Ammirato and Steward, in press).

How plants grow is, of course, still a mystery. Nevertheless, a part of the mystery involves the right kind of exogenous signals to cells applied at the right time. At this point one should take a glimpse into the future.

Earlier one noted that a great event in the last part of the 19th century was the understanding of how angiosperms reproduce, what an ovule is and what the zygote is and the role of the endosperm. A man I greatly admired at Cornell (known also to your Director, Dr. Horsfall), Professor Lester W. Sharp (1919), drew a diagram to illustrate for the

* B represents a complete basal culture medium such as that of White. CM represents the liquid endosperm of the coconut used at 10% by volume. NAA represents napthalene acetic acid at a few parts per million.

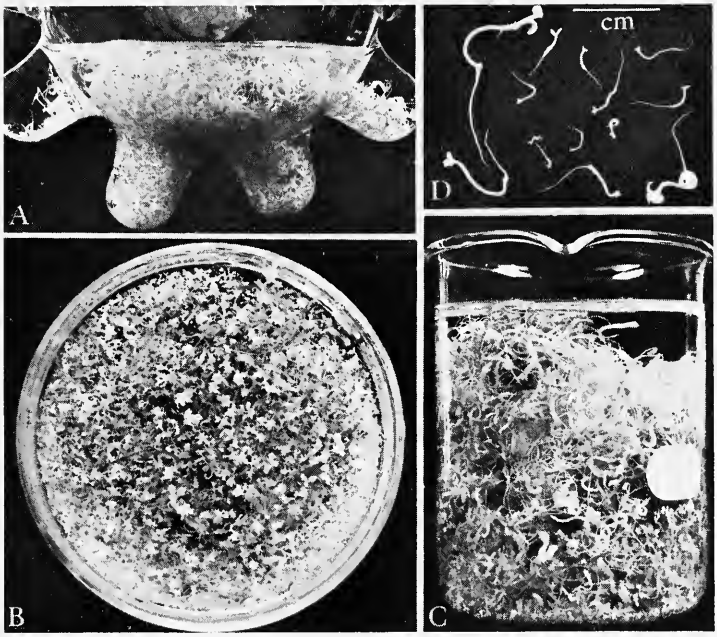


Fig. 4. Very large numbers of normal plantlets developed in liquid medium from free somatic cells: A, the culture flask with its crop of plantlets; B, a representative sample of the crop in a petri dish; C, the total crop in a 250 ml beaker; D, typical plantlets as grown.

cherry "How the Plant Produces Seed." But all the information that entered the zygote at fertilization persists in all the ordinary living cells of the plant body. Recent Nobel Prize winners have told us a great deal about how that information gets into the zygote in the first place. Nevertheless, we are still faced with major problems about how that information works itself out, and here lies one of the great tasks of the future.

Waddington, in *The Strategy of the Gene*, used an illustration which illustrates my point (Waddington, 1957). In this picture one visualizes the zygote taking off with all its information, to accomplish a marvelous journey through time and space, a journey which makes our missiles seem very primitive and wasteful organizations indeed. Waddington visualized the zygote passing over what he called "the epigenetic landscape," like a sphere in a sort of biological "pinball machine," for it could be diverted this way, or that way, to form roots and shoots, sun leaves and shade leaves, long-day plants and short-day plants; all these events being subject to "epigenetic control" in the sense that the controls are not genetic alone, for there is "something else" which over-rides the genetic information. This "something else" is what we need now to say something about.

The idea is that there are chemical signals which intervene at various points to "trip the switches," as it were, which divert and control the course of development and metabolism, so that the environmental controls are mediated chemically. A dramatic example of this is to be seen

Fig. 5. Control of metabolism (anthocyanin production) by growth promoting substances (A & B rephotographed from Kodachrome transparencies). A, red (left) and green (right) strains of *Haplopappus* cells and cell clusters as grown in rotating (1 rpm) flasks and in culture media involving different concentrations of naphthaleneacetic acid. B, typical *Haplopappus* cells and cell clusters showing some daughter cells heavily pigmented while an adjacent cell is virtually free of anthocyanin.

in *Haplopappus* cultures (Fig. 5) grown from free cells in a basal medium supplemented with coconut milk and a little naphthaleneacetic acid. Under one set of conditions the cultures may be greenish white, but by changing the naphthaleneacetic acid content concentration the cells grow about as well but they form so much anthocyanin that the culture appears almost black (Fig. 5A). The anthocyanin forming information is there all the time, but it needs an external exogenous, even chemical, stimulus to bring it into play. In intermediate conditions one may also observe situations in which even attached daughter cells, products of the division of one cell, differ in that something tells one cell to be much more pigmented than the other. Often one cell remains colorless, or even develops some chloroplasts, while the other develops the red pigment (Fig. 5B). Thus, there is a sort of "division of labor" here. In fact, some two-celled stages exhibit a very embryological form —one cell being more pointed and pink, the other rounded and colorless. When such cells are attached, they have protoplasmic connections, and some mysterious something seems to tell one cell to do one thing and the attached cell another. This is not due to asymmetries in the environment, for we have created the most symmetrical culture environment we know how to achieve. Here, however, one must recognize the role of morphology and form for, although I have the greatest respect for cellular biochemistry, I must still admit that the biochemistry alone is not in sole control of the situation. In the final analysis it is the morphology that seems to "call the shots" so that the biochemistry is "its servant, not its master."

We have grown a culture of carrot cells so green that they contained as much chlorophyll in as good chloroplasts as one would expect to find in leaf cells. But now, if you make the culture organize, one can see in these little organizing structures glimpses of bright orange carotene pigment which never occurs to this extent in the free cell cultures until they do so organize. In fact, in minute carrot plantlets, so minute that they are only observed under the microscope, although all the cells are genetically identical, they form green chloroplasts in the rounded embryonic shoot, colorless leucoplasts in their root tips, and rich red carotenoids, as in the mature carrot root, where secondary thickening begins. So all the cells have all the information, but it is not just "what the cells are" that matters, for their behavior is conditioned also by "where they are." The great task, therefore, is to translate the "where they are" into meaningful terms of biophysical and biochemical stimuli.

A very recent development in my laboratory relates again to the carrot cultures. It was always a puzzle that in any bushel of carrots from the best seedsmen in California, no two roots were exactly alike with respect to their response to the stimuli to growth. Thus the carrots have been tested before use, or each of our experiments has been done with a clone of explants obtained from a single carrot root. However, it is now clear that, over and above the genetic effects of carrot varieties and origins, there are interpretable differences between these carrot

roots. Explants from all carrots will make much growth on a medium which contains coconut milk, but the roots can be divided into groups according to the response of their explants to indoleacetic acid on the one hand and to inositol on the other. It is now known that the coconut milk (or other similar) complex comprises at least two systems which make carrot cells grow. In the one system the cell division factors *per se* interact with indoleacetic acid, and this is called System II. But another, System I, also comprises a variety of cell division factors which, in this case, interact with inositol. The two systems, I and II, can work independently so that some carrot roots respond more to one than to the other. But it is now clear that both of these systems also work through iron, and that there are also ways of linking them together, for in some way this occurs through the supply of exogenous reduced nitrogen by the use of casein hydrolysate in the medium (Steward and Degani, Ann. Bot., in press).

Using a trace element free basal medium and a trace element free coconut milk, one may also find that even when all the organic stimuli are present, but without iron, that nothing happens (c.f. Steward, Neumann and Rao, 1968; also unpublished work of Rao). In that sense, therefore, iron is a "kinin," and over and above iron the next trace element of importance in this connection is molybdenum. The lesson here is that growing cells do not, for the convenience of investigators, isolate all their attributes so that they can be thought of and studied singly. This is not how growing systems work, and if you find one that does respond solely to one factor, independently of all others, then it is usually a system whose total responses had already become severely limited. Such an example is the coleoptile of cereal grains, for all it can do is to elongate as cells expand. Indoleacetic acid, therefore, emerged as a cell elongation factor in this system. But in the context of the carrot assay and of cells that can divide as they grow IAA can be shown to be as much a cell-division factor (i.e. it behaves as a kinin) and, if all other parts of the complex are present, it in effect *keeps cells small.* Thus, we should not limit the functions of a growth regulator by the characteristics of an assay system which is adopted for the convenience of the investigator for, in so doing, we may limit nature to our own terms (Fig. 6). On the contrary, I believe that it is only by studying the system whole that we shall arrive at the complex truth.

In conclusion, therefore, consider a two-celled stage of a carrot (Steward et al., 1963, c.f. Fig. 3, p. 79) floating free, with a globular part and a more pointed part, already showing some "division of labor," for it is clear that one is going to develop toward the shoot, the other to the suspensor or the root. What is it in the marvelous biological engineering of the cells, all with the same genetic information, that tells one cell to do something so very different from the cell to which it is attached? At least one can see that the protoplasmic connections may coordinate the system. But at least we know that out of such free cells, by the hundreds and thousands, if one wishes to do so, one may now grow

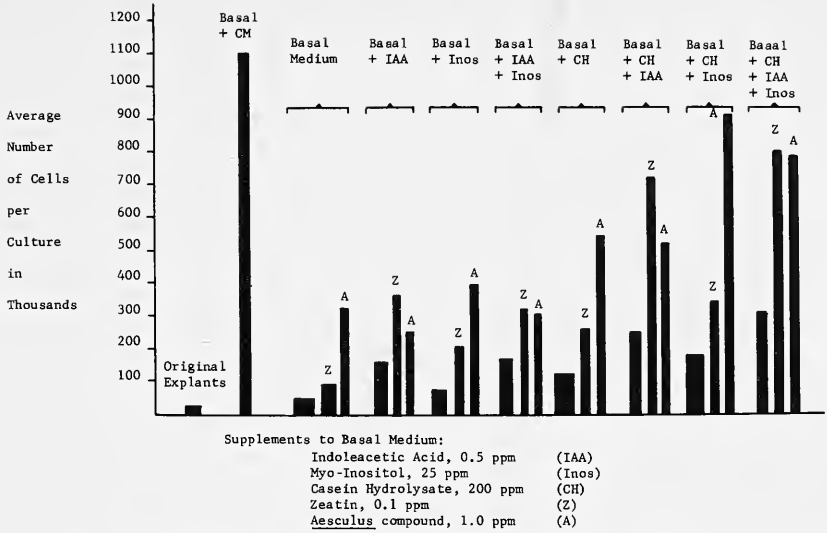


Fig. 6. Interactions of the components of systems which induce the growth of carrot explants relative to the basal medium and to the effect of coconut milk (CM): histogram height and width indicate cell number and average cell size per explant respectively.

plants in which every cell *in situ* is told what it should do, when it should, and where it should, even down to the few last red pigmented petals, in the center of the inflorescence of the Queen Anne's lace which, for some reason best known to *Daucus carota*, invariably turn pink. Although it might seem that these flowers might have dispensed with, or forgotten about this feature, when they are grown from free cells, nevertheless the red petals reappear (Steward, et al., 1964). Do you wonder, then, that I am seized with the challenge of the controls of biological organization as they are expressed in the way plants develop?

Like Johnson, whose book is celebrated, I started as a chemist and, like him, I have also written a book. His, in 1868, was called *How Crops Grow;* and mine, in 1968, is called *Growth and Organization in Plants.* This book shows that I am challenged not only by the question how plants feed nor even by what allows plants to inherit, or their cells to grow, for we can now grow their cells freely and in abundance. The great challenge now is to understand how in the morphological environment of a totipotent plant cell it is told what to do, when and where it should so that development and morphogenesis may occur in a coordinated way. These problems inevitably involve the organization of whole cells and whole plants and they raise questions of what may be termed "biological engineering"; questions which the Victorians may have seen in the terms of what they called "division of labor." But the

modern developments present a great opportunity because somatic cells of higher plants may respond to external stimuli and so harness the genetic information of their nuclei and all the machinery of their cytoplasm to grow and develop, embryologically, in ways which have not yet proved feasible for the somatic cells of higher animals. But, to understand this system at work we need to recognize, and to be able to deal with, all the complex interactions between the physiological functions and with the exogenous stimuli and the environmental factors by which the behavior and metabolism of otherwise totipotent, often autotrophic, plant cells is determined in crop plants as they grow.

ACKNOWLEDGMENTS

It goes without saying that many collaborators, shown in the published papers, were concerned with different aspects of the original work here cited. Mrs. Marion O. Mapes and Mrs. Gertrude S. Olsen assisted in converting the verbatim recorded account of the extempore lecture into this printed, illustrated version. Also, over the years, the work on which the lecture was based was supported by the National Cancer Institute and the Institute of General Medicine of the Department of Health, Education, and Welfare.

September 17, 1968

REFERENCES

Ammirato, P. V. and F. C. Steward. 1969. Indirect effects of irradiation: Morphogenetic effects of irradiated sucrose. Developmental Biology 19: 87-106.

Blakely, L. M. and F. C. Steward. 1961. Growth induction in cultures of *Haplopappus gracilis*. I. The behavior of the cultured cells. Amer. J. Bot. 48: 351-358.

Haberlandt, G. 1902. Culturversuche mit isolierten Pflanzenzellen. Sitzber. kais. Akad. Wiss., Math-naturwiss. Kl., Bd. CXI, Abt. I, 69-92.

Hales, Stephen. 1727. Vegetable Staticks: or An account of some statical experiments on the sap in vegetables: being an essay towards a natural history of vegetation. London, W. and J. Innys. 376 p.

Hales, Stephen. 1738. Statical essays: containing Vegetable Staticks, etc. The third edition, 2 vol., London, W. and J. Innys.

Johnson, S. W. 1868. How Crops Grow. Orange Judd & Co., New York.

Johnson, S. W. 1870. How Crops Feed. Orange Judd & Co., New York.

Kent, A. E. 1966. The totipotency of cultured plant cells: Its control during development and morphogenesis. Ph.D. Thesis, Cornell University, pp. 177.

Krikorian, A. D. and F. C. Steward. 1968. Water and Solutes in Plant Nutrition: With Special Reference to van Helmont and Nicholas of Cusa. BioScience 18: 286-292.

Reynolds Green, J. 1900. An Introduction to Vegetable Physiology. J. & A. Churchill, London.

Reynolds Green, J. 1909. A History of Botany (1860-1900) Being a continuation of Sachs 'History of Botany 1530-1860.' Oxford Press, 543 p.

Richards, O. W. and A. J. Kavanagh. 1945. The Analysis of Growing Form. *In* Growth and Form, Essays, p. 188-230. Ed. W. E. Le Gros Clark and P. B. Medawar. Oxford.

Sachs, J. 1875. History of Botany (1530-1860). English translation by Garnsey and Balfour. 1890. Oxford Press. 568 p.

Sharp, L. W. 1919. How the plant produces seed. *In* The Cornell Reading Course for the Farm, Lesson 144, The Plant Series. N. Y. State College of Agriculture at Cornell University, Ithaca, N. Y.

Steward, F. C. 1961. Vistas in plant physiology: Problems of organization, growth, and morphogenesis. Can. J. Bot. (Duff Memorial Volume) 39: 441-460.

Steward, F. C. 1963. The control of growth in plant cells. Scientific American 209(4): 104-113.

Steward, F. C. 1966. Physiological aspects of organization, p. 3-26. *In* E. G. Cutter (ed.), Trends in Plant Morphogenesis.

Steward, F. C. 1968. Growth and Organization in Plants. Addison-Wesley; Reading, Mass.

Steward, F. C., L. M. Blakely, A. E. Kent and M. O. Mapes. 1963. Growth and organization in free cell cultures, p. 73-88. *In* Meristems and Differentiation (from the Brookhaven symposia in biology), No. 16. Brookhaven National Laboratory, Upton, N. Y.

Steward, F. C., S. M. Caplin and F. K. Millar. 1952. Investigations on growth and metabolism of plant cells. I. New techniques for the investigation of metabolism, nutrition and growth in undifferentiated cells. Ann. Bot. 16: 57-77.

Steward, F. C. and N. Degani. 1969. Endogenous characteristics of different clones of carrot explants and their exogenous requirements for growth. Ann. Bot. 33: 615-646.

Steward, F. C., Ann E. Kent and Marion O. Mapes. 1966. The culture of free plant cells and its significance for embryology and morphogenesis, p. 113-154. *In* A. Monroy and A. A. Moscona (ed.), Current Topics in Developmental Biology. Academic Press, New York.

Steward, F. C., A. E. Kent and M. O. Mapes. 1967. Growth and organization in cultured cells: Sequential and synergistic effects of growth-regulating substances. Ann. N. Y. Acad. Sci. 144: 326-334.

Steward, F. C. and M. O. Mapes. 1963. The totipotency of cultured carrot cells: Evidence and interpretations from successive cycles of growth from phloem cells, p. 237-247. *In* T. S. Sadasivan (ed.), J. Indian Bot. Soc. XLIIA (Maheshwari commemoration volume). Bangalore Press, India.

Steward, F. C., M. O. Mapes, A. E. Kent and R. D. Holsten. 1964. Growth and development of cultured plant cells. Science 143; 20-27.

Steward, F. C., M. O. Mapes and K. Mears. 1958. Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. Amer. J. Bot. 45: 705-708.

Steward, F. C., K. H. Neumann and K. V. N. Rao. 1968. Investigations on the growth and metabolism of cultured explants of *Daucus carota*. II. Effects of iron, molybdenum and manganese on metabolism. Planta (Berl.) 81: 351-371.

Steward, F. C. and E. M. Shantz. 1955. The chemical induction of growth in plant tissue cultures. (I. Methods of tissue culture and the analysis of growth. II. The chemical nature of the growth promoting substances in coconut milk and similar fluids: The present position), p. 165-186. *In* R. L. Wain and F. Wightman (ed.), The Chemistry and Mode of Action of Plant Growth Substances. Academic Press, N. Y.

Steward, F. C., E. M. Shantz, J. K. Pollard, M. O. Mapes and J. Mitra. 1961. Growth induction in explanted cells and tissues: Metabolic and morphogenetic manifestations, p. 193-246. *In* D. Rudnick, Molecular and Cellular Synthesis (19th Growth Symposium). Ronald Press, N. Y.

Waddington, C. H. 1957. Strategy of the Genes, pp. 262. Macmillan, New York.

Wardlaw, C. W. 1955. Embryogenesis in Plants. pp. 381. Methuen, London.

How Crops Absorb Water

Paul J. Kramer

Crop production has made great progress in the century since Samuel Johnson (1868) wrote the book *How Crops Grow*. Geneticists and plant breeders have given us high yielding, disease-resistant varieties, agronomists have developed improved cultural methods and fertilization schedules, and engineers have provided better machinery for cultivating and harvesting crops. Unfortunately, however, crops are still exposed to the same hazards of weather as in 1868, and all too often the weather prevents our improved varieties from attaining their potential yields.

With modern cultural methods and varieties, crop yields in the United States are reduced more often by lack of water than by any other factor. This is true even in the humid Southeast where studies of precipitation show that droughts of sufficient severity to reduce crop yields occur nearly every year from Virginia to the southern Mississippi Valley (van Bavel and Verlinden, 1956; van Bavel, Forest, and Peel, 1957; van Bavel, 1959).

Magnitude of the Absorption Problem

The high level of turgidity required for good growth can be maintained only if water absorption keeps pace with transpiration. The magnitude of the water absorption problem for crop plants seems to have

Table 1. Transpiration ratio of several crop plants growing at Akron, Colorado, expressed as units of water transpired per unit weight of dry matter produced above ground. From Shantz and Piemeisel, 1927

Plant	1911	1912	1913
Alfalfa	1,086	657	834
Oats	639	449	617
Barley	527	443	513
Wheat	468	394	496
Corn	368	280	399
Millet	287	187	286
Sorghum	298	239	298
Evaporation in cm, April to September	122	94.3	107.5

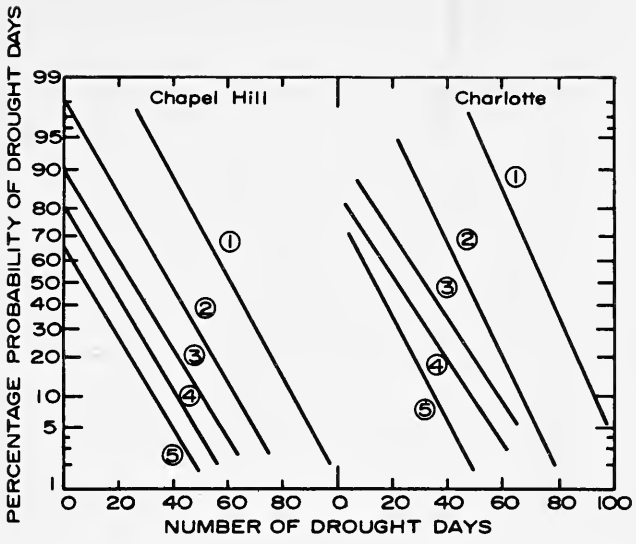


Fig. 1. The probable number of drought days at two stations in North Carolina during the period from April 1 to September 30. The figures on the ordinate give the probability of having the number of drought days indicated on the abscissa. The number on each line indicates the inches of available soil water on which the estimate is based. The probability of drought is much greater in a shallow, sandy soil holding only 1 inch of readily available water than in a deep, fine textured soil holding 5 inches. From van Bavel and Verlinden, 1956.

been understood by Johnson who cited data from Hales concerning the amount of water lost in transpiration. More modern data indicate that crop plants commonly use 250 to 500 units of water per unit of dry matter produced and Burton, Prine and Jackson (1957) reported that from 450 to over 9000 units of water were used per unit of dry matter produced by Bermuda grass grown under various conditions. Corn plants can lose more than their own weight of water on a hot, dry day and 100 times their weight in a growing season, and a crop of Illinois corn may lose 12 to 18 cm of water by transpiration and an equal amount by evaporation in a growing season.

Immediate replacement of these water losses is essential to the success of crops because growth is controlled by the internal water balance or degree of turgor existing in plants. Loss of turgor results in closure of stomata, decrease in photosynthesis, disturbance of carbohydrate and nitrogen metabolism, cessation of cell enlargement, and eventually death by desiccation. The internal water balance of plants depends on the relative rates of water absorption and water loss, and successful growth is possible only if water absorption keeps pace with water loss by transpiration.

This situation poses two important problems, (1) how are such large quantities of water absorbed, and (2) how is absorption linked to transpiration? In order to understand these problems we will discuss the absorption process and some factors which affect it. It is impossible to discuss the problems of crop water relations in detail or cite all of the important literature in this chapter. Readers are therefore referred to books by Kramer (1969), Kozlowski (1968), and Slatyer (1967) for additional information and citations.

HOW WATER IS ABSORBED

Historical Review

There has been as great a change from 1868 to 1968 in ideas about water absorption as there has been in farming methods. In 1868 ideas concerning the absorption of water had not progressed much beyond those of Hales who published his Vegetable Staticks in 1727. In 1827 de Candolle published his spongiole theory, according to which root tips sucked in water, and forced it into the roots by contraction. Johnson discarded the spongiole theory as inaccurate, and accepted the osmosis theory of Dutrochet who stated that absorption by roots is an osmotic action exercised by the membrane that bounds the young roots and root hairs. Johnson stated that osmosis occurs in the epidermis with such energy that it not only distends the cells, but causes water to filter through their walls into the xylem. This explanation probably came from the German physiologists of the 19th century, but it really explained nothing about water absorption through the roots of intact plants.

Modern plant physiology often is said to have begun with Sachs whose research was becoming well known in the 1860's. He made important contributions to plant water relations, studying the water-holding capacity of various types of soil, the effects of aeration and temperature on water absorption, and the absorbing zone of roots. He attributed root pressure to the osmotic activity of root cells and believed that loss of water by transpiration produced the pull responsible for the ascent of sap. A few years later Dixon and Askenasy demonstrated the high cohesive force in water which enables it to be pulled from the roots to the evaporating surfaces in transpiring leaves.

Two important advances bearing on plant water relations were made in the present century. One advance was the realization that water movement from cell to cell and tissue to tissue is controlled by gradients in activity or chemical potential, rather than by gradients in osmotic pressure. This concept resulted in a variety of new terms such as suction force, suction tension, and diffusion pressure deficit. Today the widely used term, diffusion pressure deficit, is being replaced by the term water potential because the latter is more in accord with current usage by physical chemists and because it permits separation of the forces affecting the chemical potential of water into their matric, osmotic, and

pressure components. Furthermore, it is equally applicable for description of the water status of cells, entire plants, and soil (Kramer, Knipling, and Miller, 1966; Slatyer, 1967).

This concept of plant water relations is shown by the following equation:

$$\Psi_{cell} = \Psi_s + \Psi_m + \Psi_p$$

where Ψ_{cell} is the potential of the water in the cell; and the other terms describe the contributions to Ψ_{cell} by solutes (Ψ_s), matric forces of water building colloids and surfaces (Ψ_m), and pressure (Ψ_p). Ψ_s and Ψ_m are negative, Ψ_p is positive unless there is a negative wall pressure, a rare occurrence. Assuming no change in cell volume or in Ψ_s (not strictly correct) and disregarding Ψ_m the relationship for cells of various turgidities can be shown as follows:

	Ψ_{cell}		Ψ_s	+	Ψ_p
Fully turgid	0	=	−20	+	(+20)
Partly turgid	−10	=	−20	+	(+10)
Flaccid	−20	=	−20	+	0

Another stimulating contribution to the field of plant water relations was the application of the analogy between flow of electrical current in a conducting system, as described by Ohm's law, and the flow of water through the soil-plant-atmosphere system. According to this concept water movement is a continuous process from soil through the plant to the atmosphere, controlled by the gradient in driving force and the resistances in the pathway. It is often described by equations such as the following:

$$\text{Water Flux} = \frac{\Delta\Psi_{soil}}{r_{soil}} = \frac{\Delta\Psi_{root}}{r_{root}} = \frac{\Delta\Psi_{stem}}{r_{stem}} = \frac{\Delta\Psi_{leaf}}{r_{leaf}} = \frac{\Psi_{leaf} - \Psi_{air}}{r_{leaf} + r_{air}}$$

This treatment is an oversimplification because the resistance to water flow through plants changes with change in driving force and flux is not directly proportional to the difference in potential. Also the change in state which occurs during transpiration is a complicating factor because the driving force for liquid water usually is expressed as pressure in bars and that for water vapor as millimeters of vapor pressure. Nevertheless, the concept is very useful because by breaking water movement into a number of steps investigators can analyze the importance to water movement of various factors in the soil, the plant, and the air in terms of their effects on the driving forces and the resistances in various parts of the system. It also encourages development of useful models, such as those of Cowan (1965) and Waggoner and Reifsnyder (1968).

The history of these two concepts shows the difficulty of getting new ideas accepted. Use of the term water potential seems to have been proposed in 1949 by Schofield at a meeting held at Rothamsted and it

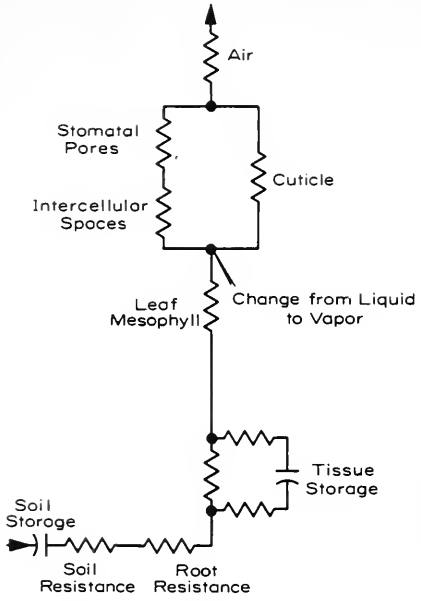


Fig. 2. Diagram showing the analogy between flow of water through the soil-plant-atmosphere continuum and flow of electricity through a system of conductors and resistances. (After Cowan, 1965.)

was used by Owen in a paper in 1952. However, it has only begun to be used extensively since 1965. Gradmann proposed in 1928 the analogy between the flow of water through plants and electricity in conductors and in 1948 van den Honert discussed it at a symposium sponsored by the Faraday Society. However, the concept was largely neglected until the present decade when it began to be used so widely that John Philip of Canberra recently warned against its misuse (Philip, 1966). Apparently a new generation of scientists must come of age in order for new ideas to be accepted.

Current Theories of Water Absorption

Water absorption occurs only when the water potential in the roots is lower than in the soil. However, the cause of the lower root water potential is different in slowly and rapidly transpiring plants, resulting in two different absorption mechanisms, active and passive absorption. In slowly transpiring plants growing in warm, moist, well aerated soil the water potential in the root xylem sap is reduced by the accumulation of solutes, causing the roots to behave like osmometers. As a result inward diffusion of water occurs and hydrostatic pressure often develops in the stele, causing root pressure and the occurrence of "bleeding" from wounds and guttation from leaves. This mechanism is often called active absorption because it depends on active accumulation of salt in the roots. However, there is no active transport of water involved, but only the active transport of salt. In fact there is no evidence of active transport of water occurring anywhere in plants.

Active absorption and the associated root pressure and guttation are very interesting phenomena. However, there is no evidence that active absorption plays any essential role in plant water relations. This is a good example of the fact that not all interesting problems are important nor are all important problems interesting. Much research has been done on active absorption because it is interesting rather than because it is important. Study of root systems is much more important, but it is dirty, dull, and uninteresting so little is done about it.

In rapidly transpiring plants the water potential is first lowered in the leaf cells by loss of water in transpiration, and this decrease in water potential is transmitted to the roots as tension in the water conducting system. Thus in rapidly transpiring plants the roots act simply as passive absorbing surfaces through which water moves by mass flow, pulled by the matric or imbibitional forces developed in the evaporating surfaces of the leaf cells. Most of the water absorbed by crop plants is absorbed passively. The passive theory of water absorption explains how the intake of water is linked to the loss of water by the continuous cohesive water columns which extend from roots to shoots in all seed plants. These continuous columns of water provide a control or feedback mechanism which links absorption to transpiration. Increased loss of water by transpiration decreases the water potential in the leaves and this decrease is transmitted through the water columns in the xylem to the roots where the steeper gradient in water potential from soil to roots causes increased water absorption. Conversely, reduced absorption decreases the water supply to the leaves and the resulting loss of turgor in leaf cells causes closure of stomata and reduction in water loss. This linkage or feedback mechanism which couples absorption to transpiration is extremely important to the survival of land plants growing in relatively dry air.

The action of this feedback mechanism is "damped" somewhat by the relatively high resistance to radial water movement into roots. As a result, on sunny days absorption often lags somewhat behind water loss resulting in removal of water from parenchyma tissue and transient midday deficits. These deficits are replaced by absorption during the night as long as water is available in the soil.

FACTORS AFFECTING WATER ABSORPTION

The rate at which water is absorbed is controlled principally by the rate of transpiration. However, it is affected by various other internal and external factors, including the extent and permeability of roots, the availability of soil water, soil temperature, aeration, and concentration of the soil solution.

The driving force for absorption of water is the difference in water potential between the bulk soil and the root surface and between the root surface and the xylem sap. The rate of water flow with a given

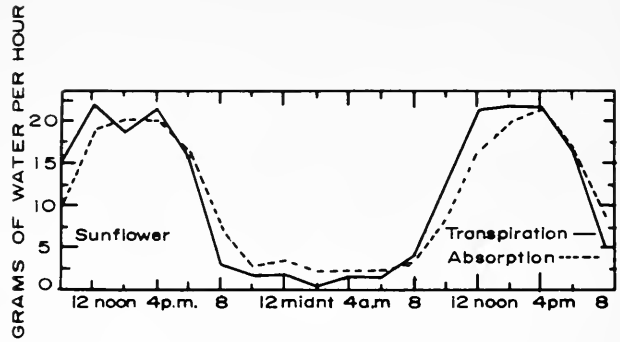


Fig. 3. The lag of absorption behind transpiration in auto-irrigated sunflower plants exposed to bright sun. This lag is caused chiefly by high resistance to water movement through roots. It is responsible for temporary midday water deficits such as that indicated by the reduced transpiration from 12 to 2 pm on day 1. From Kramer, 1937.

driving force depends on the resistance to flow in the soil and the roots. These relationships can be shown by the following equation.

$$\text{Absorption} = \frac{\Psi_{soil} - \Psi_{root\ surface}}{r_{soil}} = \frac{\Psi_{root\ surface} - \Psi_{root\ xylem}}{r_{root}}$$

The various factors affecting water absorption operate either by affecting the driving force from soil to root xylem or the resistance to flow through the soil or the root, or both. Soil texture and hydraulic conductivity affect resistance to water movement through the soil while aeration, temperature, and degree of suberization of roots affect absorption chiefly by increasing root resistance. Drying soil reduces the water potential gradient from soil to roots but it also increases soil resistance and sometimes root resistance. We will now discuss some of the factors affecting absorption in more detail.

Importance of Root Systems

Nothing is more important to the success of crop plants than the vigorous growth of their root systems because the larger the soil mass occupied by root systems the larger the volume of water available to them and the longer plants can survive without replenishment of soil water. An example is sorghum. Miller (1916) attributed the fact that sorghum is more drought resistant than corn to the presence of nearly twice the number of fine roots found in corn. Slatyer (1955) further reported that sorghum has a better root system than cotton or peanuts. Although it has not been proven, it seems probable that more of the difference in drought resistance among species and varieties of crop

plants is related to differences in depth and branching of roots than to any other factor. Thus plant breeders might well pay more attention to development of extensive root systems and farmers ought to keep in mind the necessity of providing a suitable environment for root growth.

There has been some question whether roots at a distance from a plant are as efficient as those nearby (Davis, 1940). However, most of the evidence indicates that roots several meters from a tree can absorb water effectively (Veihmeyer and Hendrickson, 1938; Wiggans, 1936). Reimann, Van Doren and Stauffer (1946) reported an instance where by early August Illinois corn had exhausted all available water in the upper meter of soil, but was growing well on water absorbed from below one meter. McWilliam and Kramer (1968) found that plants of *Phalaris tuberosa* L. survived when the water potential in the top meter of soil was below −15 bars, but died when the roots were cut at a depth of one meter. Laboratory experiments showed that both water and ^{32}P were absorbed from a depth of more than one meter and translocated to the shoots. Apparently some plants can absorb enough water to replace transpiration losses if one-fourth to one-half of their root system is in soil above the permanent wilting percentage (Taylor, Blaney, and McLaughlin, 1934).

The pattern of water absorption from soil is different for rapidly growing annuals and established perennials. Annual plants first absorb the soil water near the base of the plant and if no water is added the volume of drying soil enlarges as the roots extend out laterally and vertically. Unless water is added, absorption occurs from increasing depths as the season progresses and continual expansion of root systems is essential to survival. Trees and other perennial plants have a different pattern of water absorption because they start the growing season with a root system which already occupies a large volume of soil. In the spring such plants may begin immediately to extract water from throughout the soil mass occupied by roots, but because the root concentration usually is higher near the trunk and in the surface soil, soil water content often is reduced first near the plants before much is absorbed from deeper horizons.

The Absorbing Zone. There is some difference of opinion concerning the location of the absorbing zone of roots. The classical view is that water and salt are absorbed through the root hair zone. However, most root systems consist of roots in various stages of differentiation, ranging from newly formed tips to fully matured secondary roots which have lost their epidermis and cortex and are enclosed in a layer of suberized tissue. Johnson stated that suberized roots do not function in absorption of water and modern textbook writers appear to hold the same opinion. They emphasize the importance of very young roots in absorption, and ignore the possibility that measurable absorption occurs through older, suberized roots. No doubt in young, rapidly growing annuals most water and salt absorption occurs through young roots.

Table 2. Relative permeability of grape roots of various ages and conditions to movement of water and ^{32}P. The base rate for water uptake through terminal roots is 0.001 ml/hr/cm^2 under a pressure gradient of 50 cm of mercury. From Queen, 1967

Type of Root	Relative Permeabilities Water	^{32}P
Roots of current season		
Terminal 8 cm, elongating, unsuberized, unbranched	1	1
Unsuberized, but bearing elongating branches	155	75
Main axis and branches partially suberized, elongation ceased	65	35
Roots of preceding season		
Heavily suberized main axis with short suberized branches	0.2	0.04
Heavily suberized main axis without branches	0.02	0.02

However, in perennials and particularly in trees, only a small fraction of the total root system is unsuberized and sometimes in the winter no unsuberized roots can be found. Nevertheless, evergreens continue to transpire on sunny days in the winter, and under these conditions considerable quantities of water must be absorbed through suberized roots. Often less than one per cent of the root surface is unsuberized (Kramer and Bullock, 1966), and the large surface area of suberized roots compensates for their lower permeability. It seems probable that suberized roots play a significant role in both water and salt absorption.

Views concerning the path followed by water moving radially across roots also have changed since the time of Johnson. It formerly was assumed that all water moves from vacuole to vacuole across the cortical cells. However, it now seems probable that considerable water movement may occur in the cell walls, at least as far as the endodermis, because there is less resistance to water movement in cell walls than through the cytoplasm. Much more needs to be learned concerning the relationship between root structure and salt and water absorption.

Other Functions of Roots. Roots have other functions in addition to serving as absorbing surfaces. Little attention is given to their role in anchorage, yet this is very important to trees and tall crops such as corn. Their importance as storage organs also should be remembered. Even more neglected is their synthetic activity. Inorganic nitrogen compounds are converted into organic compounds in the roots of many species (Bollard, 1960; Kretovich, 1965). The synthesis of nicotine in the roots of tobacco and other alkaloids in the roots of *Datura* and *Atropa* also are well known. Now there is evidence that both cytokinins

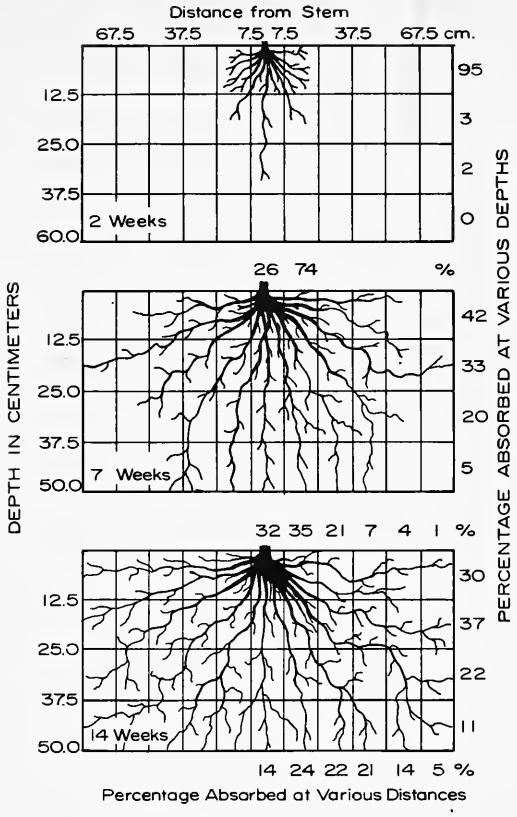


Fig. 4. The relationship between root extension and mineral absorption by growing corn plants. Root extension is equally important for water absorption unless the soil is rewetted frequently by rain or irrigation (After Hall, et al. 1953).

and gibberellins are synthesized in the roots (Jones and Lacey, 1968; Kende, 1965; Skene, 1967).

It seems possible that soil water stress may reduce the supply of cytokinin sufficiently to disturb nitrogen metabolism in the leaves. Perhaps the reduced growth of shoots on roots subjected to low temperature, inadequate aeration, or water stress results partly from reduction in the supply of growth regulators and organic nitrogen to the shoot, as well as reduced water supply. The importance of roots as a source of growth regulators and other organic compounds certainly deserves further investigation.

The effects on neighboring plants of substances escaping from roots also may be important. For example black walnut (*Juglans nigra* L.)

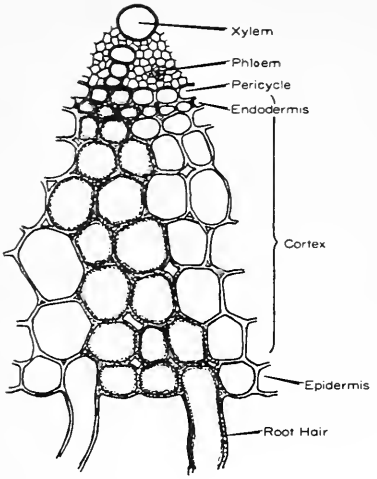


Fig. 5. The pathway of radial movement of water across roots. It formerly was assumed that most of the water moved from vacuole to vacuole, but it is now believed that much water movement occurs through cell walls, at least as far as the endodermis. Resistance to movement across the cortical tissue is responsible for the absorption lag seen in Fig. 3.

and quackgrass (*Agropyron repens* L.) are said to produce substances toxic to nearby plants. The various organic substances which normally escape from roots must have considerable effect on the microbial population which in turn affects the root environment. There is also some evidence that products formed during decomposition of roots and other plant parts sometimes are toxic to other root systems. However, this interesting topic lies outside of the boundary of this paper and readers are referred to the symposium edited by Baker and Snyder (1965) and papers such as those by Woods (1960) and Overland (1966) for further information and references.

Availability of Soil Water

The availability of soil water to plants depends on whether or not it moves to roots as rapidly as it is absorbed. If it does not move rapidly plant water deficits develop, resulting in reduced growth and yield. The rate of water movement to roots depends on the steepness of the water potential gradient from soil to roots and the hydraulic conductivity of the soil.

Root Water Potential. Roots of slowly transpiring plants often behave as osmometers and their water potential depends on the concentration of solutes in the xylem sap. The osmotic potential of the xylem sap seldom is lower than −2 bars, hence active water absorption by slowly transpiring plants or detopped root systems seldom occurs from soils or solutions with a water potential lower than −2 or −3 bars. In rapidly transpiring plants the tension in the transpiration stream may reduce the root water potential to −15 or −20 bars on sunny days, or even −50 to −100 bars in plants subjected to severe water deficits.

Soil Water Potential. The total water potential of soil can be described by the following equation.

$$\Psi_{soil} = \Psi_m + \Psi_s + \Psi_p + \Psi_g$$

Ψ_m is the matric potential, Ψ_s the solute or osmotic potential, Ψ_p is the pressure potential, and Ψ_g the gravitational potential. The pressure potential is important only in saturated soils and the gravitational potential can be neglected in respect to absorption. The osmotic potential is so small in soils of humid regions that the total soil water potential is controlled by the matric potential. Only in arid regions and occasionally in heavily fertilized soils does Ψ_s become low enough to limit plant growth. As the soil dries the matric potential decreases rapidly, decreasing the steepness of the water potential gradient from the bulk soil to the roots and slowing down water movement to the roots. When the soil water potential falls below the minimum potential attainable in roots water absorption ceases.

Hydraulic Conductivity of Soils. Decrease in soil water content is accompanied by decrease in hydraulic conductivity because the number and size of the channels through which water can move decreases. As a result less water is moved by a given gradient in water potential (Gardner, 1960, 1965) in soil near the permanent wilting percentage than in soil near field capacity. Also, coarse textured soils have a lower water conductivity than fine textured soils at the same water potential.

In view of these facts it appears that the availability of soil water to plants depends chiefly on the rate of absorption required to replace transpiration losses, the hydraulic conductivity of the soil, and the root density. Cowan (1965) and Gardner and Ehlig (1962) indicate that the resistance to water movement in soils with a water potential lower than -1 or -2 bars usually is the limiting factor for absorption. However, Newman (private communication) surveyed the data on root density in the literature and concluded that for most crop plants root density is so great that the soil resistance would not become a limiting factor until the soil water content falls to approximately the permanent wilting percentage. He found root densities under grasses as high as 1000 cm per cm^2 of soil surface, under cereals of 100 to 500 cm/cm^2, and under trees 5 to 110 cm/cm^2. In soils with moderate conductivities and a root density of 100 cm/cm^2 soil water potential must fall to about -25 bars before soil resistance becomes limiting. However, at a root density of 10 cm/cm^2 of soil surface soil resistance becomes limiting at a soil water potential of only -5 bars. These conclusions are based on a maximum rate of transpiration of 0.1 mm per hour per unit of soil surface. They seem to be compatible with the estimate of Gardner (1960) that water might move toward roots over a distance of up to 4 cm with sufficient speed to be physiologically useful. Where there is a shallow water table significant upward water movement may occur over distances of one or two meters.

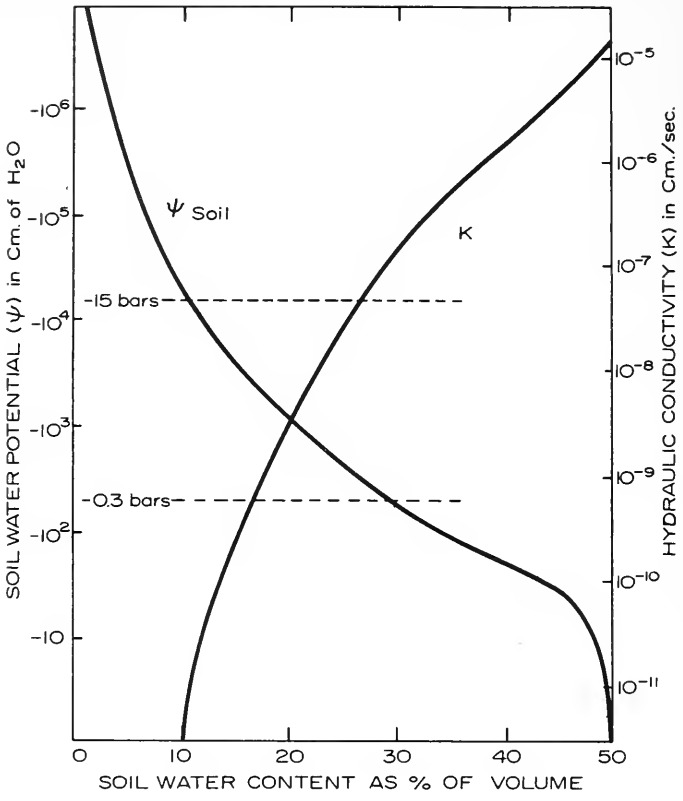


Fig. 6. The decrease in hydraulic conductivity (K) and soil water potential (ψ) which accompany decrease in soil water content (After Slatyer, 1967).

Soil Temperature

It has been known since the time of Hales that water absorption is reduced by low temperature and that there are large differences among species in their reactions to cold soil. Schroeder (1939) found that cold soil and cold water caused injury to greenhouse tomatoes and Cameron (1941) reported that orange trees in California often wilt during cold weather. Irrigation with cold water is injurious to rice, but beneficial to potatoes (Raney and Mihara, 1967). Water absorption by collards (kale) is little affected at a temperature which causes wilting of cotton or cucurbits and white pine is less affected than loblolly pine (Kramer, 1969).

Low temperature reduces water absorption chiefly because it increases resistance to water movement through roots. The increased root resistance results from an actual decrease in root permeability and

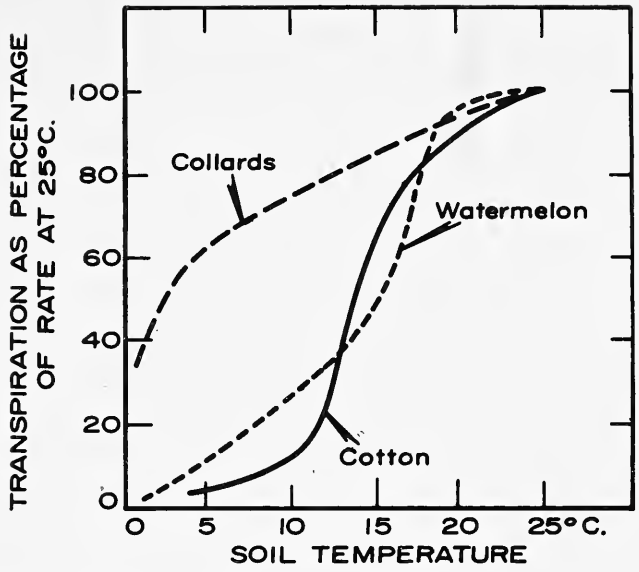


Fig. 7. Effect of soil temperature on water absorption by three species of plants, as measured by rate of transpiration. Collards thrive in cold soil, cotton and watermelons in warm soil. From Kramer, 1942.

an increase in viscosity of water (Kramer, 1940; Kuiper, 1964). Other factors such as decreased root growth and metabolism and decreased hydraulic conductivity of the soil are believed to be of secondary importance.

Soil Aeration

Deficient aeration also reduces water absorption by increasing the resistance to water movement through roots. The effects of decreased root permeability can be seen as wilting of the shoots within less than an hour after root systems are flooded, or the soil air is displaced with carbon dioxide or nitrogen. The long term effects of deficient aeration are much more complex, involving decreased growth of roots, interference with their synthetic functions, and eventually death of part or all of the root system (Kramer, 1969).

It seems probable that chronic oxygen deficiency exists in many compact soils, and probably one beneficial result of cultivation of some soils is to improve aeration.

Concentration of Soil Solution

In humid regions the salt concentration of soil seldom is high enough to reduce water absorption. However, in arid regions accumulation of

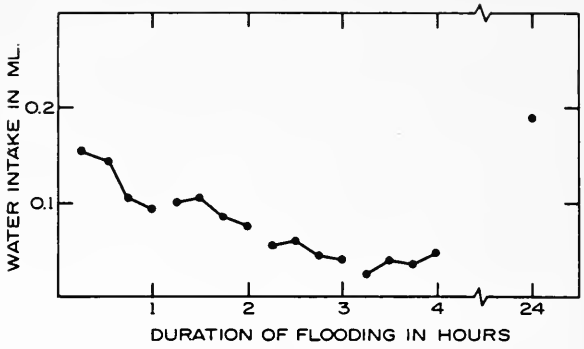


Fig. 8. Effect of saturating the soil with water for various lengths of time on the water flow through tobacco root systems subjected to a gradient of 0.2 bar. Root resistance increased and flow decreased rapidly during the first three hours. The increase in flow after 24 and 48 hours was caused by injury to the roots and they were dead in 6 days. Kramer and Jacksen, 1954.

an excess of salt often limits plant growth, and the problem occasionally develops in greenhouse soils. The classical explanation was that the presence of salt lowers the soil water potential and decreases absorption. However, in long term experiments it has been found that when salt is added gradually to the root medium plants accumulate it until their osmotic potential is lowered to about the same extent as that of the substrate in which they are growing (Eaton, 1942; Slatyer, 1961). Thus the reduction in soil osmotic potential is balanced by an equivalent reduction in plant water potential (Table 3). The following equations show what occurs in similar plants growing in a nutrient solution with an osmotic potential (Ψ_s) of -0.75 bars (A) and in a nutrient solution

Table 3. Effect of osmotic potential of culture solution on osmotic potential of plant sap. From Eaton, 1942

Osmotic potential of culture solution in bars / Species	−0.72	−2.52	−6.0
	Difference between osmotic potential of solution and plant sap.		
Milo	10.3	10.8	11.1
Alfalfa	13.0	12.6	10.4
Cotton	13.1	11.8	9.7
Tomato	8.8	8.3	8.2
Barley	9.2	12.4	14.7
Sugar beet	12.8	13.8	15.0
Average difference	11.2	11.6	11.5

Fig. 9. Effect of a compacted layer of soil on root penetration by rape plants (*Brassica napus*). Left, undisturbed soil with compacted soil layer about 25 cm below the surface. Right, even penetration of roots into soil loosened by cultivation. Restriction of root penetration was caused by mechanical resistance because aeration was not limiting below the compacted layer (De Roo, 1961). Courtesy of H. C. De Roo, The Connecticut Agricultural Experiment Station.

to which enough NaCl is added to decrease Ψ_s to -5.75 bars (B). In the latter situation the plants accumulate salt until the osmotic potential of their roots is reduced as much as that of the root substrate, hence the gradient in osmotic potential ($\Delta\Psi_s$) from solution to roots is approximately the same in both solutions.

	Ψ_s of nutrient solution	Potentials in Root Cells						$\Delta\Psi_s$
		Ψ_s		Ψ_p		Ψ_w		
A	-0.75	-10	+	9.25	=	-0.75		9.25
B	-5.75	-15	+	9.25	=	-5.75		9.25

Plants which accumulate salt continue to absorb water and sometimes are even more succulent than low salt plants. However, the reduction in growth caused by added salt often is almost proportional to the re-

duction in osmotic potential. It appears that we must look to the effects of reduced cell water potential and relative turgor pressure rather than to reduced water absorption for an explanation of salt injury. The cells are now subjected continuously to a much lower water potential and although the turgor pressure or pressure potential is little changed it is relatively lower than before salt was added. Perhaps instead of being in the soil the physiological drought is in the protoplasm. As Repp, McAllister, and Wiebe (1959) suggest, the ability of plants to grow in saline soils probably is limited chiefly by the ability of their protoplasm to tolerate the salt which they absorb. Plants with low tolerance fail to survive because of injury to their protoplasm and interference with metabolic processes rather than from desiccation. This view is supported by the fact that when salt stress is removed growth does not return to normal (Greenway, 1962) but when soil water stress is removed growth returns to normal or even exceeds the normal rate for a time (Gates, 1955; Miller, 1965). It must be kept in mind that a high concentration of salt reduces root growth and root permeability, and this may reduce water absorption significantly. However, reduced water absorption seems to be distinctly secondary to the effects of the lowered water potential caused by salt accumulation. The problem of growth inhibition by salt obviously requires more research.

FUTURE RESEARCH IN PLANT WATER RELATIONS

It seems probable that the need for research on plant water relations will continue to increase as the need for more food results in cultivation of more semiarid land where drought always is a problem. Furthermore, there is increasing realization that water deficits often reduce yields in humid areas. Only a few of the many problems which might be investigated will be mentioned.

Improved Root Systems

The first possibility is to improve the absorbing system. Much of the apparent drought resistance found in crop plants results from deep, much branched root systems. More attention should be paid by plant breeders to the development of root systems which penetrate deeply and branch profusely. Perhaps agronomists ought to pay more attention to providing soil conditions suitable for good root development (de Roo, 1961). As mentioned earlier, vigorous root systems probably are important as synthetic organs as well as absorbing organs.

Reduction of Transpiration

Numerous attempts have been made to reduce transpiration by applying substances to leaves which either reduce cuticular transpiration or cause stomatal closure. It is assumed that stomatal closure will reduce

transpiration more than it reduces uptake of carbon dioxide (Slatyer and Bierhuizen, 1964). The work of Slatyer and Bierhuizen (1964), Waggoner and Bravdo (1967), and Zelitch (1965) are examples of this approach. The literature on the chemical reduction of transpiration was reviewed by Gale and Hagan (1966).

Perhaps plant breeders should investigate the possibility of producing leaves with thick cutin and very responsive stomata which will conserve water during short droughts. Reduction in energy absorption by modification of leaf color or leaf arrangement might also deserve consideration. The most efficient crop plant is pineapple which uses only about one tenth as much water per unit of dry matter as most other crops (Ekern, 1965). This plant has crassulacean metabolism which means that it absorbs CO_2 at night when the stomata are open and converts it into carbohydrate during the day. The stomata are largely closed during the day so water loss is very low. If we could introduce this kind of metabolism into some more useful kinds of plants it would greatly increase their ability to survive and even grow with a minimum of water.

Increased Tolerance of Dehydration

We also should pay more attention to differences in ability to tolerate dehydration. There are great differences in the ability of various kinds of plants to endure dehydration, remain green, and resume growth when rewatered. Sorghum is better than corn in this respect and Coastal Bermuda is better than many other grasses. However, the reasons for their differences are not known. If the reasons for dehydration resistance were known perhaps we could introduce it into other species and varieties. Increased tolerance of dehydration and prompt recovery on rewatering would be particularly useful in humid climates where droughts usually are of short duration.

Increased Salt Tolerance

Large areas of land in dry climates are unproductive because of their high salt content and much irrigated land has been rendered useless or nearly so by salt accumulation. Furthermore, much water is unsatisfactory for irrigation because of its high salt content. If we had a better understanding of why excess salt injures some kinds of plants more than others it might help in developing varieties with increased tolerance of salt. Even a small increase in salt tolerance would make it possible to use large areas of land which cannot be cultivated profitably at present.

SUMMARY

For successful crop growth water absorption must keep pace with water loss from plants. Most of the water uptake by crop plants occurs by passive absorption in which the roots act as a passive absorbing surface through which water is pulled into plants.

The absorption of water depends on the steepness of the water potential from soil to roots and the resistances to water movement through soils and roots. Rate of transpiration is the principal factor controlling the water potential of roots, and soil water content and salt content control the soil water potential. Decreasing soil water content also increases resistance to water movement through soil and sometimes increases root resistance.

Deficient aeration and low temperature reduce water absorption chiefly by increasing resistance to water movement through roots and secondarily by decreasing root growth. Increased salt content in the soil decreases soil water potential and rate of root growth and increases root resistance.

Because of the slow movement of water in drying soil the extent and amount of branching of root systems has important effects on the success of plants. It is believed that the entire root system functions as an absorbing surface, not merely the unsuberized root hair zone. There also is increasing evidence that the synthetic activities of roots may contribute both organic nitrogen and growth regulators to the shoots.

Much additional research is needed on ways of increasing the efficiency of water use and the drought resistance of crop plants. Among the interesting possibilities are:

1. Breeding for deep, much branched root systems and providing soil conditions which stimulate deep rooting.
2. Reduction in rate of transpiration by chemical treatments to close stomata or by breeding for more responsive stomata and thicker cutin. The crassulacean metabolism of pineapple results in extraordinary efficiency in water use.
3. Search for factors which enable plants to endure short periods of dehydration and resume growth with minimum permanent injury when rewatered.

October 21, 1968

BIBLIOGRAPHY

Baker, K. F., and W. C. Snyder (eds.). (1965). Ecology of soil-borne plant pathogens, prelude to biological control. Berkeley, Univ. of California Press.

van Bavel, C. H. M. 1959. Drought and water surplus in agricultural soils of the lower Mississippi valley area. U.S. Dept. Agr. Tech. Bull. 1209.

van Bavel, C. H. M., L. A. Forrest, and T. C. Peele. 1957. Agricultural drought in South Carolina. South Carolina Agr. Exp. Sta. Bull. 447.

van Bavel, C. H. M., and F. J. Verlinden. 1956. Agricultural drought in North Carolina. North Carolina Agr. Exp. Sta. Tech. Bull. 122.

Bollard, E. G. 1960. Transport in the xylem. Ann. Rev. Plant Physiol. 11: 141-166.

Burton, G. W., G. M. Prine, and J. E. Jackson. 1957. Studies of drought tolerance and water use of several southern grasses. Agron. Jour. 49: 498-503.

Cameron, S. H. 1941. The influence of soil temperature on the rate of transpiration of young orange trees. Proc. Am. Soc. Hort. Sci. 38: 75-79.

Cowan, I. R. 1965. Transport of water in the soil-plant-atmosphere system. J. Appl. Ecol. 2: 221-239.

Davis, C. H. 1940. Absorption of soil moisture by maize roots. Bot. Gaz. 101: 791-805.
De Roo, H. C. 1961. Deep tillage and root growth. Connecticut Agr. Exp. Sta. Bull. 644.
Eaton, F. M. 1942. Toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. 64: 357-399.
Ekern, P. C. 1965. Evapotranspiration of pineapple in Hawaii. Plant Physiol. 40: 736-739.
Gale, J., and R. M. Hagan. 1966. Plant antitranspirants. Ann. Rev. Plant Physiol. 17: 269-282.
Gardner, W. R. 1960. Dynamic aspects of water availability to plants. Soil Sci. 89: 63-73.
Gardner, W. R., and C. F. Ehlig. 1962. Some observations on the movement of water to plant roots. Agron. Jour. 54: 453-456.
Gates, C. T. 1955. The response of the young tomato plant to a brief period of water shortage. I. The whole plant and its principal parts. Australian J. Biol. Sci. 8: 196-214.
Gradmann, H. Untersuchungen uber die Wasserverhältnisse des bodens als grundlage des pflanzenwachstums. Jahrb. wiss. Bot. 69: 1-100. 1928.
Greenway, H. 1962. Plant responses to saline substrates. I. Growth and ion uptake of several varieties of Hordeum during and after sodium chloride treatment. Australian J. Biol. Sci. 15: 16-38.
Hall, N. S., W. F. Chandler, C. H. M. van Bavel, P. H. Reid, and J. H. Anderson. 1953. A tracer technique to measure growth and activity of plant root systems. North Carolina Agr. Exp. Sta. Tech. Bull. 101.
Honert, T. H. van den. 1948. Water transport as a catenary process. Faraday Soc. Discussions No. 3, 146-153.
Johnson, S. W. 1868. How crops grow. Orange Judd Co., New York.
Jones, O. P. and H. J. Lacey. 1968. Gibberellin-like substances in the transpiration stream of apple and pear trees. J. Expt. Bot. 19: 526-531.
Kozlowski, T. T. (ed.). 1968. Water deficits and plant growth. Academic Press. New York.
Kramer, P. J. 1937. The relation between rate of transpiration and rate of absorption of water in plants. Amer. Jour. Bot. 24: 10-15.
Kramer, P. J. 1940. Root resistance as a cause of decreased water absorption by plants at low temperatures. Plant Physiol. 15: 63-79.
Kramer, P. J. 1942. Species differences with respect to water absorption at low soil temperatures. Amer. Jour. Bot. 29: 828-832.
Kramer, P. J. 1969. Plant and soil water relationships: a modern synthesis. McGraw-Hill Book Company. New York.
Kramer, P. J. and W. T. Jackson. 1954. Causes of injury to flooded tobacco plants. Plant Physiol. 29: 241-245.
Kramer, P. J. and H. C. Bullock. 1966. Seasonal variations in the proportions of suberized and unsuberized roots of trees in relation to the absorption of water. Am. J. Bot. 53: 200-204.
Kramer, P. J., E. B. Knipling, and L. N. Miller. 1966. Terminology of cell-water relations. Science 153: 889-890.
Kretovich, W. L. 1965. Some problems of amino acid and amide biosynthesis in plants. Ann. Rev. Plant Physiol. 16: 141-154.
Kuiper, J. C. 1964. Water uptake of higher plants as affected by root temperature. Meded. Landb. Hogesch. Wageningen 63: 1-11.
McWilliam, J. R. and P. J. Kramer. 1968. The nature of the perennial response in Mediterranean grasses. I. Water relations and summer survival in *Phalaris*. Australian J. Agr. Res. 19: 381-395.
Miller, E. C. 1916. Comparative study of the root systems and leaf areas of corn and the sorghums. Jour. Agr. Res. 6: 311-332.

Miller, L. N. 1965. Changes in radiosensitivity of pine seedlings subjected to water stress during chronic gamma irradiation. Health Physics 11: 1653-1662.
Overland, L. 1966. The role of allelopathic substances in the "smother crop" barley. Amer. Jour. Bot. 53: 423-432.
Philip, J. R. 1966. Plant water relations: some physical aspects. Ann. Rev. Plant Physiol. 17: 245-268.
Queen, W. H. 1967. Radial movement of water and ^{32}P through suberized and unsuberized roots of grape. Ph.D. Dissert. Duke University.
Raney, F. C. and Y. Mihara. 1967. Water and soil temperature. pp. 1024-1036. in R. M. Hagan et al. (eds.). Irrigation of agricultural lands. Amer. Soc. Agron. Madison.
Reimann, E. G., C. A. Van Doren, and R. S. Stauffer. 1946. Soil moisture relationships during crop production. Proc. Soil Sci. Soc. Amer. 10: 41-46.
Repp, G. I., D. R. McAllister, and H. H. Wiebe. 1959. Salt resistance of protoplasm as a test for salt tolerance of agricultural plants. Agron. Jour. 51: 311-314.
Schroeder, R. A. 1939. The effect of root temperature upon the absorption of water by the cucumber. Univ. Missouri Res. Bull. 309: 1-27.
Shantz, H. L. and L. N. Piemeisel. 1927. The water requirement of plants at Akron, Colorado. J. Agr. Res. 34: 1093-1190.
Skene, K. G. M. 1967. Gibberellin-like substances in root exudate of *Vitis vinifera*. Planta 74: 250-262.
Slatyer, R. O. 1955. Studies of the water relations of crop plants grown under natural rainfall in northern Australia. Australian J. Agr. Res. 6: 365-377.
Slatyer, R. O. 1961. Effects of several osmotic substrates on water relations of tomato. Australian J. Biol. Sci. 14: 519-540.
Slatyer, R. O. 1967. Plant-water relationships. Academic Press, London and New York.
Slatyer, R. O. and J. F. Bierhuizen. 1964. The influence of several transpiration suppressants on transpiration, photosynthesis, and water-use efficiency of cotton leaves. Australian J. Biol. Sci. 17: 131-146.
Taylor, C. A., H. F. Blaney, and W. W. McLaughlin. 1934. The wilting-range in certain soils and the ultimate wilting point. Amer. Geophysical Union Trans. 15: 436-444.
Veihmeyer, F. J. and A. H. Hendrickson. 1938. Soil moisture as an indication of root distribution in deciduous orchards. Plant Physiol. 13: 169-177.
Waggoner, P. E. and B. A. Bravdo. 1967. Stomata and the hydrologic cycle. Proc. Nat. Acad. Sci. U.S. 57: 1096-1102.
Waggoner, P. E. and W. E. Reifsnyder. 1968. Simulation of the temperature, humidity and evaporation profiles in a leaf canopy. J. Appl. Met. 7: 400-409.
Wiggans, C. C. 1936. The effect of orchard plants on subsoil moisture. Proc. Am. Soc. Hort. Sci. 33: 103-107.
Woods, F. W. 1960. Biological antagonisms due to phytotoxic root exudates. Bot. Rev. 26: 546-569.
Zelitch, I. 1965. Environmental and biochemical control of stomatal movement in leaves. Biol. Rev. 40: 463-482.

Control of Photosynthesis by Oxygen

Martin Gibbs

Samuel Johnson never quoted his own work, from which I conclude that he was either very modest or probably did very little original research. But he had great insight. In *How Crops Grow* he recognized the importance of photosynthesis. In *How Crops Feed* (1870) he noted that there are two opposite changes in plants one called fixation of carbon, the other called respiration. He pointed out that these opposite changes cannot take place at the same point but must proceed in different organs, or cells, or in different parts of the same cells and that they tend to counterbalance each other in their effects on the atmosphere surrounding the plant. Although it sounds simple enough today it took great insight to recognize the fact that the process in which oxygen was consumed probably went on in a different organelle from that in which oxygen was involved.

In 1920 (29) Warburg made the observation that the rate of release of O_2 by illuminated *Chlorella* was inhibited by O_2. He compared O_2 evolution when O_2 constituted from 2% to 100% of the gaseous phase and thus made the interesting discovery that the photosynthetic mechanism was being "poisoned" by the O_2 it had released. This effect on photosynthesis (carbohydrate synthesis), now commonly referred to as the "Warburg Effect" is reminiscent of the "Pasteur Effect," the inhibition of glycolysis (carbohydrate breakdown) by O_2.

The depression of photosynthesis by O_2 has been observed in a wide variety of photosynthetic organisms including algae, bryophytes, gymnosperms and angiosperms. Turner and Brittain (27) have summarized the results of the considerable amount of work on the Warburg effect in algae.

Some Characteristics of the Warburg Effect

McAlister and Myers (20) were the first to record O_2 inhibition of photosynthesis in higher plants. In a species of *Triticum,* they observed a 25% inhibition of CO_2 fixation by 21% O_2 when contrasted to a control of 100% N_2. Extensive comparative studies of O_2 inhibition have been recorded by Turner, Todd and Brittain (26) and by Björkman (6).

A detailed study of this inhibition was made by Tamiya and co-

workers (21, 22, 24). Tamiya and Huzisige (24) were the first to note that the effect of O_2 was rapidly and completely reversible when O_2 was replaced by N_2. This reversibility has been confirmed by others and is an important characteristic which must be considered when possible mechanisms to explain the inhibition are sought. Another characteristic of this phenomenon is that the magnitude of the inhibition by O_2 is affected drastically by CO_2 concentration. For instance, Tamiya and Huzisige (24) measured the inhibition of 100% O_2 in CO_2 concentrations from 1700 μM to 1μM and found that this increased from 5% to 85%. Coombs and Whittingham (10) found similar results.

Benson (5) reported that the incorporation of $^{14}CO_2$ into glycolic acid was altered drastically by O_2. He found that a reduction of O_2 partial pressure from 20% to 1% in *Scenedemus,* diminished the concentration of glycolic acid tenfold. In *Chlorella* photosynthesizing in $^{14}CO_2$, Bassham and Kirk (4) as well as Coombs and Whittingham (10) noted that a change from 21% O_2 to 100% O_2 caused a sharp decrease in sugar mono- and diphosphates labeling while there was a concomitant increase in the labeling of glycolate and glycine. These data were based on isotopic percentage incorporation.

Essentially all work hitherto has been performed with the intact cell. The experiments described here have been carried out with the isolated spinach chloroplast and primarily with the inhibition of CO_2 fixation by O_2. First, it was necessary to learn whether the characteristics of this inhibition previously described in intact cells of algae and higher plants could be duplicated in isolated chloroplasts. The second aspect was to determine with isolated chloroplasts an explanation for the depressing influence of O_2 on photosynthesis.

Results

Sensitivity of Chloroplast Preparations to Oxygen

The first experiments were designed to ascertain the degree of sensitivity of the chloroplast system to O_2. The means and range of results from five experiments to test this point are plotted in Fig. 1. The great sensitivity is noticeable from the fact that 50% of $^{14}CO_2$ uptake is inhibited by 1.5% O_2 and 90% inhibition occurs in 21% O_2 under these conditions.

Kinetics of Carbon Dioxide Fixation as Influenced by Bicarbonate and Oxygen Concentrations

Another well documented property of the Warburg effect in intact organisms is that the magnitude of inhibition is related inversely to the bicarbonate concentration (24). A similar property was observed in the intact chloroplast and the progress curves for CO_2 assimilation

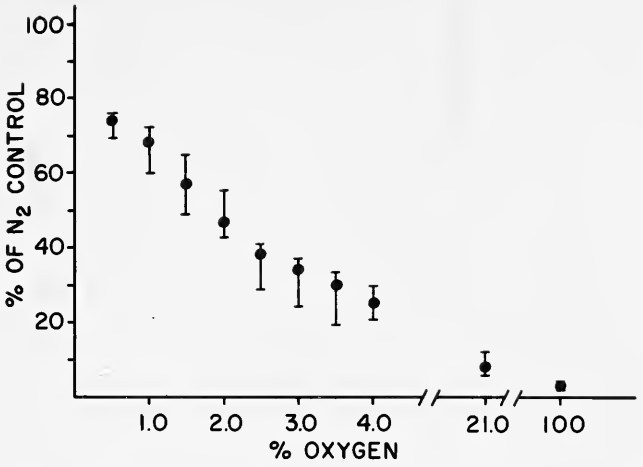


Fig. 1. Effect of O_2 on CO_2 fixation in intact chloroplasts. The data are presented as the means and ranges of 5 experiments. The bicarbonate concentration was 1.5 mM. Each experiment was run for 15 min.

are recorded in Fig. 2. Ideally, it would be desirable to plot progress curves for each bicarbonate concentration for a single chloroplast preparation. The rate of decay of CO_2 fixation activity makes it mandatory to complete an individual experiment within a short period and therefore this limits the number of determinations. For this reason a fresh chloroplast preparation was used for each set of progress curves presented in Fig. 2. Despite the spread of maximum rates of photosynthesis, the interactions between O_2 and bicarbonate concentrations within a single preparation were reproducible and when necessary were verified in a number of separate experiments. At the highest bicarbonate level used (13 mM) the result was the complete elimination of O_2 inhibition (Fig. 2A). At the lowest level (1 mM) for which data are shown (Fig. 2D), 21% O_2 and 100% O_2 brought about an inhibition of about 90%. By suitable manipulations of the bicarbonate concentration a variety of inhibitions by a particular O_2 concentration was observed.

Reversibility of Oxygen Inhibition

The data presented in Fig. 3 indicate that the Warburg effect in isolated chloroplasts is reversible. The rapidity of recovery depends upon bicarbonate concentration. The higher levels of bicarbonate (5 mM and 2.5 mM) would have recovered even in 100% O_2 with a slightly longer lag period. The lower levels (1.0 and 0.5 mM), however, would not have recovered unless the switch of atmosphere had been made.

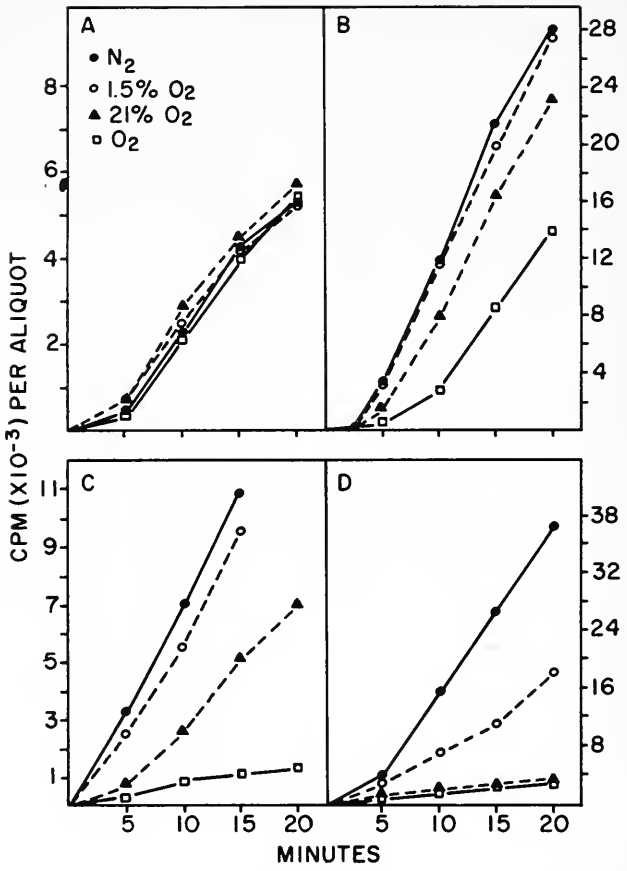


Fig. 2. Influence of O_2 and HCO_3 concentrations on CO_2 fixation in intact chloroplasts. In each case gas phases were 100% N_2 (●), 1.5% O_2 + 98.5 N_2 (○), 21% O_2 + 79% N_2 (▲) and 100% O_2 (□). Bicarbonate concentrations and maximum rates of CO_2 fixation were: A, 13 mM bicarbonate, 42 μmoles per mg chl per hr; B, 10 mM bicarbonate, 94 μmoles per mg chl per hr; C, 4 mM bicarbonate, 35 μmoles per mg chl per hr; D, 1 mM bicarbonate, 31 μmoles per mg chl per hr.

Influence of Sugar Phosphates on Oxygen Inhibition

The induction period of CO_2 assimilation in isolated chloroplasts first observed by Bamberger and Gibbs (2) and studied only under completely anaerobic conditions was shown to be eliminated or reduced by the addition of a variety of sugar phosphates such as fructose 1,6-diphosphate and ribose 5-phosphate. As Fig. 4 shows, the addition of 1 mM ribose 5-phosphate eliminated the inhibition due to 100% O_2. Similar data were obtained with fructose 1,6-diphosphate.

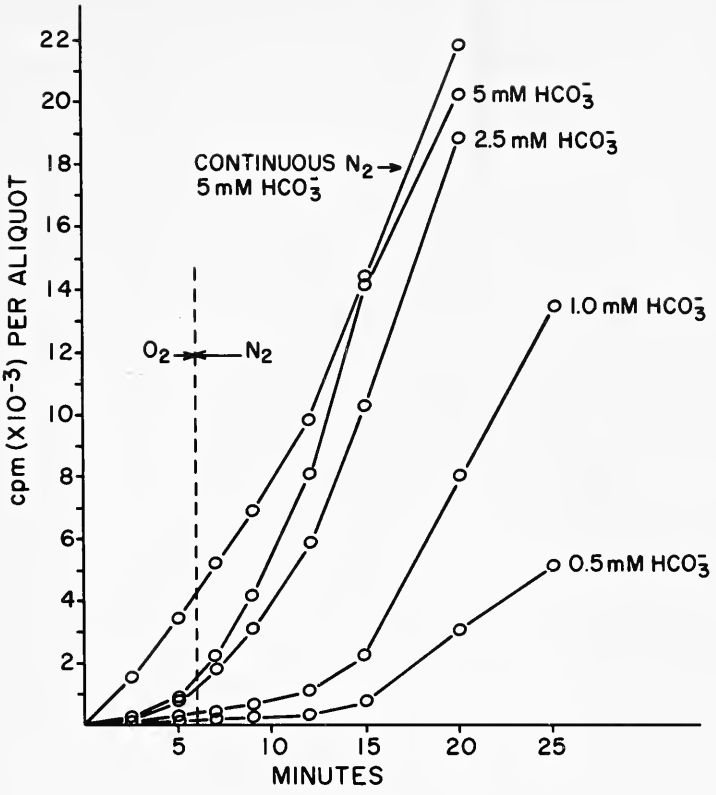


Fig. 3. The influence of changing from 100% O_2 to 100% N_2 on CO_2 fixation at several bicarbonate concentrations. One tube was maintained under continuous N_2. The others, after 6 min of photosynthesis under 100% O_2 were changed to 100% N_2. The maximum rate of photosynthesis was 37 μmoles per mg chlorophyll per hr.

Effect of Reducing Compounds

Inhibition by O_2 has been envisaged to occur at the enzymic level of the photosynthetic carbon reduction cycle (28). If an enzyme were inactivated by a photooxidative process, reducing agents such as cysteine or reduced lipoic acid might counteract this impairment. That these compounds are effective to some extent in overcoming inhibition in 1.5% O_2 may be seen in Fig. 5. It was found that dithiothreitol was also effective but reduced lipoic acid was consistently the best of the thiol compounds. The most effective concentration of these reducing agents was approximately 0.5-1 mM. The dithiol compounds, at comparable concentrations, were more effective than the monothiol compounds.

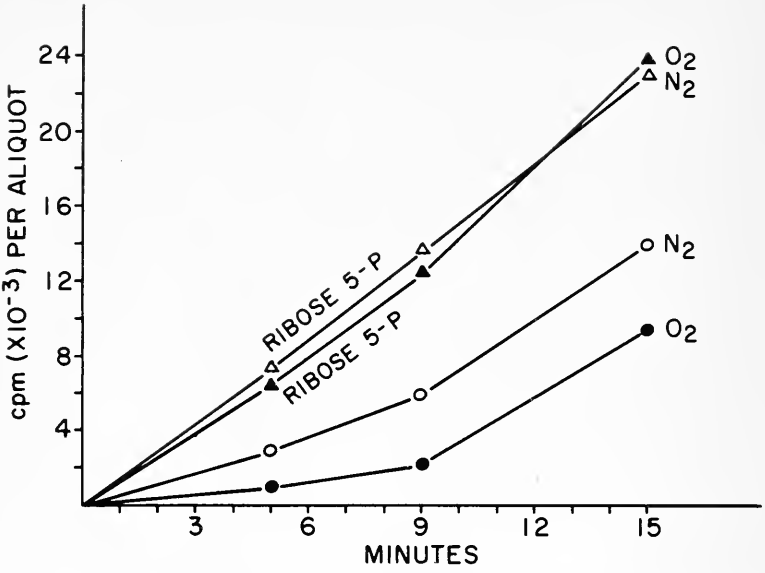


Fig. 4. The influence of 1 mM ribose 5-phosphate on CO_2 fixation in N_2 and O_2. The maximum rate of CO_2 fixation was 63 μmoles per mg chlorophyll per hr. O_2 (●); N_2 (O); O_2 and ribose 5-phosphate (▲); N_2 and ribose 5-phosphate (△).

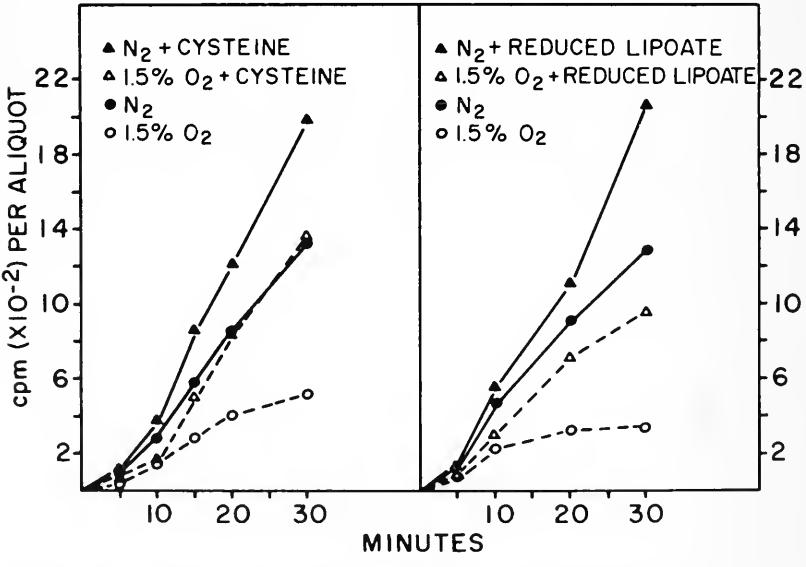


Fig. 5. The effect of 1 mM cysteine and 0.25 mM reduced lipoate on CO_2 fixation in N_2 and O_2. The maximum rate of CO_2 fixation was 64 μmoles per mg chlorophyll per hr.

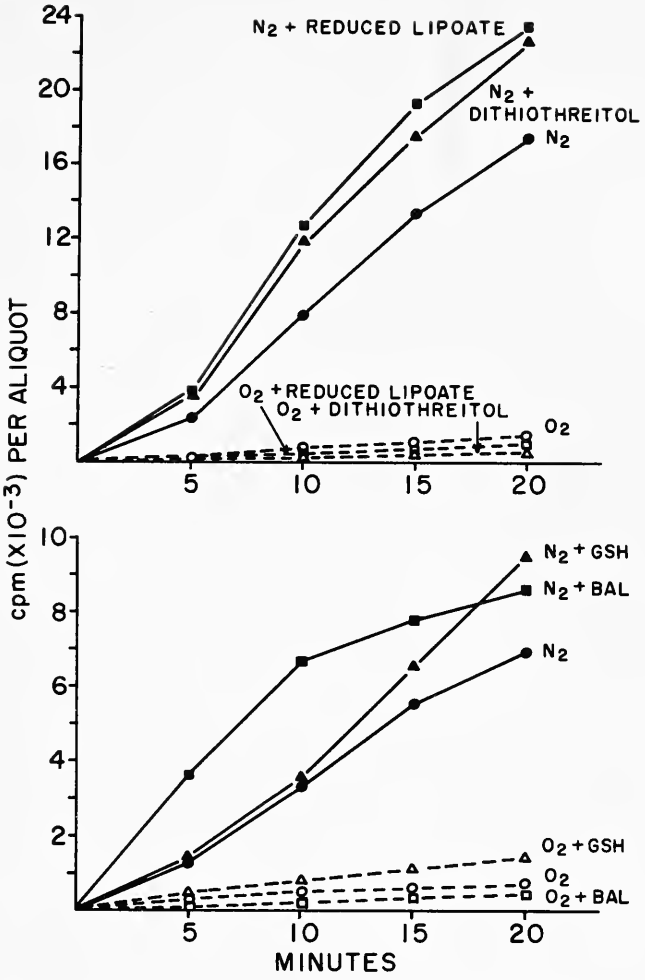


Fig. 6. The effect of several thiol compounds on $^{14}CO_2$ fixation in 100% N_2 and 100% O_2. The concentration of reduced glutathione (GSH) was 1 mM, BAL (2,3-dimercaptopropanol) 0.5 mM, dithiothreitol 0.5 mM, and reduced lipoate 0.5 mM. The maximum rate of CO_2 fixation was 100 μmoles per mg chlorophyll per hr.

Although they were effective in O_2 partial pressures up to 21%, the reduced thiol compounds were not effective in 100% O_2. This is illustrated in Fig. 6 where a variety of thiols inhibited in 100% O_2 while stimulating CO_2 fixation under N_2. This appeared to be due to the oxidation of the thiol by 100% O_2 to a form which inhibited photosynthesis.

Table 1. Effect of O_2, N_2 and light on the activity of ribulose 5-phosphate kinase
Chloroplasts were allowed to carry out photosynthesis for the times listed. After the designated period, ribulose 5-phosphate activity was determined by following the formation of ADP via pyruvate kinase and lactic acid dehydrogenase.

Conditions		*Specific Activity μmoles per mg chlorophyll per hr*		
		N_2	O_2	O_2
2 min	Dark	....	....	$O_2 \rightarrow N_2$
6 min	Dark	34	20	34
5 min	Light	106	70	76
10 min	Light		$O_2 \rightarrow N_2$	100
		100	62	

Influence of Oxygen on Enzyme of the Photosynthetic Carbon Reduction Cycle

Attention was focussed on ribulose 5-phosphate kinase (Table 1) fructose 1,6-diphosphate aldolase (Table 2) and the DPN- and TPN-linked glyceraldehyde 3-phosphate dehydrogenases (Table 2). These enzymes are known to have thiol groups associated with their activity. All activities were measured directly in chloroplast preparations after a stated period of photosynthesis. Aldolase was unaffected by O_2. The inactivation

Table 2. Effect of oxygen on DPN and TPN dependent glyceraldehyde 3-phosphate dehydrogenases and aldolase
Samples were removed at the designated time intervals and assayed for enzyme activity. Cysteine was added to glyceraldehyde 3-P dehydrogenase assay mixture 7 min prior to start of assay. Bicarbonate concentration was 1.25 mM.

Oxygen Conc.	Time	*Glyceraldehyde 3-P Dehydrogenase* TPN	DPN	TPN + 0.1 mM Cysteine	*Aldolase*
%	*Min*	*μmoles substrate per mg chl per hr*			
0 (N_2)[1]	0	79	33	186	22
"	5	74	31	219	19
"	15	84	19	298	20
"	30	56	19	242	21
100[2]	0	93	22	186	24
"	5	56	19	204	22
"	15	56	17	219	19
"	30	28	14	139	23

[1] CO_2 fixation rate was 2.7 μmoles per mg chlorophyll per hr.
[2] CO_2 fixation rate was 0.2 μmoles per mg chlorophyll per hr.

of both glyceraldehyde 3-phosphate dehydrogenases was slow but in time considerable. Inactivation was reversed to some extent by cysteine.

The specific activity of ribulose 5-phosphate kinase was lower in O_2 than N_2 after a period of photosynthesis. In both atmospheres, the specific activity of the enzyme rose during photosynthesis.

Influence of Oxygen on the Photosystem

Interference with the formation of assimilatory power (reduced pyridine nucleotide and ATP) will influence the rate of photosynthesis. Warburg in his initial paper (29) suggested that the role of O_2 might be to reoxidize a photochemical product and more recently, this point of view has been studied intensively by Miyachi, Tamiya and coworkers (21, 22).

When TPN photoreduction and its coupled photophosphorylation were measured in N_2 or in 100% O_2, no difference was observed in their rates (Table 3). Usually these experiments are performed in high concentrations (1 mM or more) of TPN and in the presence of saturating amounts of ferredoxin. Therefore, experiments were conducted down to 0.5 μM TPN. Experiments were performed in the presence and absence of added

Table 3. Effect of TPN concentration on the rate of photoreduction in N_2 and O_2
Measurements were made in a dual wavelength recording spectrophotometer with $\lambda_1 = 403$ mμ and $\lambda_2 = 340$ mμ. Incident light was 650 mμ. Experiments were made in anaerobic cuvettes and were evacuated and filled several times with the appropriate gas before the measurements were made. The reaction mixture contained tris-HCl pH 7.8, 50 μmoles; NaCl 45 μmoles; $MgCl_2$ 12 μmoles; K_2HPO_4 — KH_2PO_4 (pH 7.8) 6 μmoles; ADP 6 μmoles. A saturating amount of spinach ferredoxin was added. Chloroplasts were added to a final concentration of 20 μg. Final volume was 3.0 ml.

Oxygen Concentration	TPN Concentration	TPN Reduced
%	*μM*	*μmoles per mg chl per hr*
0 (N_2)	.....	4
"	0.5	11
"	1.0	17
"	5.0	38
"	10.0	46
"	100.0	68
100	.....	4
"	0.5	13
"	1.0	19
"	5.0	31
"	10.0	38
"	20.0	59

Table 4. Effect of O_2 concentration and ferredoxin on photophosphorylation in the absence of TPN

The chloroplast fragments were washed twice to remove endogenous TPN.

	ATP Formed	
O_2 Conc.	Ferredoxin 7.5 units/ml	Ferredoxin 30 units/ml
%	*μmoles per mg chlorophyll per hr*	
0 (N_2)	8.6	30.8
0.5	37.7	78.2
1.5	45.2	104.0
21.0	69.0	118.5
100.0	78.1	136.5
Dark 21.0	1.0	1.0

ferredoxin with similar qualitative results, although the rates were much lower when ferredoxin was not present.

It can be demonstrated that there is some component in the electron transport chain which is readily oxidizable by O_2 in the absence of TPN. Table 4 shows the rate of "pseudocylic" photophosphorylation (that is photophosphorylation in high ferredoxin concentrations and in the absence of TPN) as it is affected by O_2 partial pressure. The great increase in phosphorylation between zero and 0.5% O_2 indicates the great O_2 sensitivity of at least one component of the electron transport.

Products of Photosynthesis. Influence of O_2 on $^{14}CO_2$ Incorporation

The effect of O_2 partial pressure on the incorporation of $^{14}CO_2$ into the products of photosynthesis is shown in Table 5. The results show: (a) lower bicarbonate concentrations result in a large increase in the percentage of isotope incorporated into glycolate (irrespective of O_2 concentration) while triose phosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) and glyceric acid 3-phosphate decreased; (b) higher O_2 concentrations also increased the glycolic acid fraction. This process proceeded at the expense of all other products, which decrease. Under conditions of high O_2 and low bicarbonate, glycolic acid was almost the only labeled product.

Products of Photosynthesis. Influence of 3,-(3,4-dichlorophenol)-1,-dimethylurea (DCMU) and Ascorbic Acid

There is some evidence and speculation that the production of glycolate depends upon light and photosystem II in particular. As glycolate synthesis appeared to be closely involved with the Warburg effect, the influence of DCMU, an inhibitor of photosystem II, was tested for its effect on the formation of the products of photosynthesis. The results

Table 5.
Chloroplasts were prepared by the method of Jensen and Bassham. Samples were removed and the products were separated by descending paper chromatography. The activity was measured directly on the paper by means of a gas flow counter fitted with a cellophane window.

Oxygen conc.	Fixation Rate of CO_2	Time	*Percentage of Total Fixation*						
			Insoluble[1]	Sugar-diP[2]	Hexose-P[3]	Pentose-P[4]	Triose-P[5]	Glycerate 3-P	Glycolate
%	*μmoles per mg chl per hr*	*Min*							
High Bicarbonate, 4.0 mM									
0 (N_2)		5	15.5	21.8	3.6	12.4	17.1	21.8	7.8
0	28	15	25.3	11.5	7.4	19.2	11.3	17.9	7.6
1.5		5	27.6	17.0	1.8	14.8	13.8	17.3	8.1
1.5	26	15	25.2	11.4	8.6	7.9	14.2	20.7	8.2
21.0		5	15.2	11.6	8.0	17.9	15.2	12.5	19.6
21.0	18	15	15.8	7.7	....[6]	16.3	20.0	12.7	27.6
100		5	11.4	19.0	2.3	15.3	14.5	12.2	25.2
100	5	15	7.7	6.2	2.8	8.0	10.8	6.2	52.3
Low Bicarbonate, 0.4 mM									
0 (N_2)		5	11.4	19.0	2.3	15.3	14.5	12.2	25.0
0	30	15	7.4	16.2	....	13.4	38.3	8.1	16.8
1.5		5	15.3	25.0	7.3	14.6	12.5	12.4	7.6
1.5	28	15	13.3	8.6	4.0	13.3	29.0	10.9	19.2
21.0		5	5.0	17.5	....	20.0	15.0	20.2	22.5
21.0	5	15	7.7	10.2	6.0	10.2	17.0	10.3	38.6
100		5	.....	.....	....	.....	49.0	9.0	41.0
100	2	15	.....	.....	....	.....	28.0	12.0	60.0

[1] Polyglucan-heating this material in 1 N HCl yielded only glucose.
[2] Fructose 1,6-diP and ribulose 1,5-diP.
[3] Fructose 6-P and glucose 6-P.
[4] Mostly ribose 5-P.
[5] Dihydroxyacetone-P.
[6] No detectable activity.

of this experiment appear in Table 6. In this experiment, 10 mM ascorbate increased 2-fold the rate of CO_2 fixation but increased 12-fold the accumulation of radioactivity in glycolate. On addition of DCMU, the diminution of total fixation was paralleled by a similar decrease in

Table 6. Effect of ascorbate (10^{-2} M) and DCMU (2.5×10^{-7} M) at a rate-limiting level of CO_2 and under nitrogen on photosynthesis products after 30 minutes

CPM per 0.1 ml Reaction-Mixture

	Total	PGA	Triose-P[1]	Hexose-P[2]	Sugar-diP[3]	Glycolate	Insoluble[4]
Control	9,580	1,810	5,940	290	440	670	430
Ascorbate	16,820	1,120	6,180	310	880	7,930	400
DCMU	2,610	280	1,680	90	270	110	180
DCMU & Ascorbate	12,500	1,630	7,380	500	1,090	1,010	880

[1] Dihydroxyacetone-P.
[2] Fructose 6-P and glucose 6-P.
[3] Fructose 1,6-diP and ribulose 1,5-diP.
[4] Polyglucan-heating this material in 1 N HCl yielded only glucose.

all compounds. In the presence of DCMU, ascorbate restored $^{14}CO_2$ fixation to roughly the control level (plus ascorbic acid) but there was a substantial decrease in the amount of isotope located in glycolic acid.

Discussion

The work presented here is considered as convincing evidence that the isolated chloroplast system manifested the characteristics of the Warburg effect previously observed in intact organisms. The most notable of these were an increased inhibition of photosynthesis by O_2 at the lower bicarbonate concentrations, a decrease in the effect of O_2 by increasing concentrations of bicarbonate and the rapid reversibility when an aerobic environment was replaced by an anaerobic one. Clearly the main aspects of this effect are associated with the chloroplast and these aspects at least can be studied successfully in an isolated spinach preparation.

This discussion is divided into two major sections: (1) aspects of the work associated with the possible role of O_2 in the light-driven phase of photosynthesis; and (2) the role of O_2 in the carbon metabolism of photosynthesis.

Warburg Effect and the Activity of the Photochemical Act

A number of theories presented to explain the Warburg effect have envisaged that one or more intermediates of the photosystem may be produced in diminished quantities under O_2. The two significant products of the photochemical act are thought to be TPNH and ATP. In this study, the rate of photoreduction of TPN down to 0.5 μM TPN was identical under N_2 and O_2 (Table 3). A K_m value with respect to TPN of 5 μM was observed which is in close agreement with previously

reported values (17). This value is roughly 1/20 the concentration of pyridine nucleotides reported to be in spinach chloroplasts (15) and is suggestive of a flow of electrons to pyridine nucleotide under normal environmental conditions. Nonetheless, a loss of electrons from reduced ferredoxin to O_2 is possible (see Table 4). Therefore, under conditions favorable for a Warburg effect such as high light intensity and rate-limiting concentrations of CO_2, the level of TPN might be depleted where reduced ferredoxin may accumulate and then react with molecular O_2 resulting in a "pseudocyclic" type photophosphorylation (12). If the photogeneration of ATP and TPNH were impaired, it follows that the regeneration of the carbon acceptor for CO_2 would be diminished. Photosynthesis would, in time, be blocked unless compounds such as hexose and pentose phosphates located in the cytoplasm were transported into the chloroplasts. There is fragmentary evidence that traffic of intermediates common to glycolysis and photosynthesis occurs between cytoplasm and chloroplast (2, 9, 19). This interaction could account for the reported lack of stoichiometry between O_2 and CO_2 in weak light (23).

Taking into consideration the data reported in Tables 3 and 4 and the observation of Latzko and Gibbs (unpublished) that O_2 does not normally influence the level of ATP in photosynthesizing isolated chloroplasts, it seems reasonable to conclude that the photogeneration of ATP and TPNH is not impaired decisively by O_2 to the extent of interfering with the rate of CO_2 fixation.

Enzymes of the Photosynthetic Carbon Reduction Cycles

Turner et al. (28) have submitted evidence that the DPN- and TPN-linked glyceraldehyde 3-phosphate dehydrogenases in extracts are sensitive to partial pressures of O_2 and that inactivation is delayed by cysteine. In the present study, O_2 lowered the activities of ribulose 5-phosphate kinase (Table 1) and the dehydrogenases of glyceraldehyde 3-phosphate (Table 2). However, the specific activities of these enzymes were higher than the rates of CO_2 fixation. If inactivated by O_2, a mechanism has to be present in the chloroplast for the immediate reversibility of the Warburg effect. While reagents such as reduced lipoate and cysteine are effective in protecting the isolated enzyme, it is not likely that similar compounds constitute an *in vivo* activating mechanism. In contrast to sugar phosphates (Fig. 4) and high concentrations of CO_2 (Fig. 2), these reagents did protect the chloroplast against 1.5% O_2 (Fig. 5) but not against 100% O_2 (Fig. 6).

The data recorded in Table 1 show that at least with respect to ribulose 5-phosphate kinase, a mechanism is present in the chloroplast capable of maintaining this enzyme. Furthermore, the specific activity was higher in chloroplasts carrying out photosynthesis in N_2 than in O_2. The photosystem must be active since increase in activity of the kinase was prevented by DCMU, an inhibitor of photosystem II (18). These find-

ings are reported in more detail elsewhere (18) and suggest that a mechanism is present in the spinach chloroplast perhaps similar to that described by Hatch and Turner (14) for controlling aerobic inhibition of glycolysis in pea seeds. The light activation of fructose 1,6-diphosphatase by spinach chloroplast may be responsive to a similar mechanism (8). There is little doubt that chloroplasts possess a mechanism sustaining activities of the enzymes catalyzing the reduction of CO_2 to carbohydrate but it is unlikely to account for the observed rapid reversibility of the Warburg effect (Fig. 3).

Influence of Oxygen on Photosynthetic Carbon Metabolism

When affecting photosynthesis, oxygen appears to have its greatest influence on chloroplast carbon metabolism. It changes the level of intermediates of the photosynthetic carbon reduction cycle and particularly of glycolic acid (Table 5).

Some theories attempt to account for the Warburg effect in terms of a drainage of carbon from the photosynthetic carbon reduction cycle in the form of glycolic acid, resulting in a limiting amount of CO_2 acceptor (4, 10). The data presented here tend to provide new evidence for this point of view. Any means, whether it be high O_2 or low CO_2, that favors the formation of glycolic acid inhibits photosynthesis. On the other hand, introduction of carbon into the cycle either in terms of high CO_2 or an intermediate of the photosynthetic carbon reduction cycle, eliminates the depressing influence of O_2. Evidence has accumulated that certain intermediates of the cycle such as glycerate 3-P, fructose 1,6-diP and ribose 5-P but not glucose 6-P and fructose 6-P can readily be transported into the chloroplast (2, 9, 19). Depending on the levels of these compounds in the cytoplasm and if this movement into the chloroplast were of sufficient rate to balance-off the drainage of glycolic acid, measurements of the Warburg effect would be variable and may account for some of the discrepancies in the literature (for instance compare Björkman (6) who did not observe a Warburg effect with Bassham and Kirk (4) who recorded a 30% inhibition of photosynthesis by air).

Glycolate is certainly a key to an understanding of the Warburg effect. Two mechanisms have been suggested for the synthesis of glycolate. One group envisages that the two carbon acid is formed by the oxidation of α-β-dihydroxyethylthiamine pyrophosphate derived from a pentulose, hexulose or heptulose monophosphate. The transfer reaction is catalyzed by transketolase but the source of oxidizing power is not evident. Coombs and Whittingham (10) have suggested H_2O_2 formed during the oxidation of ferredoxin by O_2 while Hess and Tolbert (16) have favored an oxidant produced by photosystem II. Our data presented in Table 6 whereby CO_2 assimilation was inhibited by DCMU and restored by ascorbate is considered as evidence that an oxidant is

derived prior to the DCMU site of inhibition. Current thinking places this site somewhere on the reducing side of photosystem II. Experiments have been performed in order to determine the source of the carbon skeleton of glycolic acid. Bradbeer and Anderson (7) demonstrated a very low rate of 0.22 μmole glycolate per mg chlorophyll per hr when intact chloroplasts were incubated with fructose 6-phosphate. Using a similar system, Ellyard (11) recorded rates in the order of 0.18 μmole glycolate per mg chlorophyll per hr. In this laboratory, Shain (unpublished) has now increased the rate 10-fold by incubating broken chloroplasts with very high concentrations of transketolase. In intact chloroplasts glycolate synthesis from CO_2 can reach 20 μmoles per mg chlorophyll per hr.

The second proposed mechanism is that glycolate results from the direct condensation of two C_1 units with the help of reducing power generated in the light. It was first proposed by Tanner et al. (25) and later supported by Warburg and Krippahl (30) and Zelitch (32). At low bicarbonate, glycolate is the dominant compound. This could be the result of the high K_m (20 mM) of ribulose 1,5-diphosphate carboxylase with respect to CO_2 and a flow of carbon directly to glycolate. Experiments hitherto carried out with the chloroplast favor the sugar phosphate hypothesis. However, the direct condensation hypothesis cannot be discounted since the $C_1 + C_1$ reaction may occur in the cytoplasm rather than in the chloroplast. A comparable system is the conversion of two molecules of CO_2 to acetic acid by the obligate anaerobe, *Clostridium thermoaceticum* (3). The data of Anderson and Fuller (1) obtained with *Rhodospirillum rubrum* may be interpreted in terms of a direct condensation to CO_2 to glycolate.

Conclusion

There is convincing evidence that glycolate is produced during photosynthesis in some way as a response of an aerobic environment. Following the lead of Zelitch (31), current thinking has centered about glycolate as the substrate for photorespiration. Photorespiration like photosynthesis responds to an increasing partial pressure of O_2. Plants whose photosynthesis is not depressed by O_2 apparently do not photorespire (13). Certainly, there is little doubt that an intermediate common to the Warburg effect and photorespiration is glycolate. An important and unanswered question deals with the inability of certain plants such as maize and sugar cane to form substantial quantities of glycolic acid. Finally, there is little doubt that the Warburg effect is as complex as its counterpart in carbohydrate breakdown, the Pasteur effect.

Acknowledgment

These data are derived from the efforts of Peter W. Ellyard, Erwin Latzko, Zvi Plaut, Yerachmiel Shain, and Bernice Schacter. This in-

vestigation was aided by grants from the United States Atomic Energy Commission and the National Science Foundation. Y. Shain is a postdoctoral trainee supported by Training Grant GM-1586 from the Institute of General Medical Sciences, United States Public Health Service.

November 18, 1968

LITERATURE CITED

1. Anderson, L. and R. C. Fuller. 1967. Photosynthesis in *Rhodospirillum rubrum*. II. Photoheterotrophic carbon dioxide fixation. *Plant Physiol., 42:* 491-96.
2. Bamberger, E. S. and M. Gibbs. 1965. Effect of phosphorylated compounds and inhibitors on CO_2 fixation by intact spinach chloroplasts. *Plant Physiol., 40:* 919-26.
3. Barker, H. A. and M. D. Kamen. 1945. Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. *Proc. Nat. Acad. Sci.* U.S., *31:* 219-25.
4. Bassham, J. A. and M. Kirk. 1962. The effect of oxygen on the reduction of CO_2 to glycolic acid and other products during photosynthesis by *Chlorella*. *Biochem. Biophys. Res. Comm., 9:* 376-80.
5. Benson, A. A. 1950. Carboxylation reactions of photosynthesis. In: CO_2 Assimilation Reactions in Biological Systems. Ed., M. Gibbs, Brookhaven Conference Report 70 (C-13) 119-38.
6. Björkman, O. 1966. The effect of oxygen concentration on photosynthesis in higher plants. *Physiol. Plant., 19:* 618-33.
7. Bradbeer, J. W. and C. M. A. Anderson. 1967. Glycolate formation in chloroplast preparations. In: Biochemistry of Chloroplasts, Vol. II, ed. T. W. Goodwin. Academic Press, London and New York.
8. Buchanan, B. B., P. C. Kalberer and D. I. Arnon. 1967. Ferredoxin-activated fructose diphosphatase in isolated chloroplasts. *Biochem. Biophys. Res. Comm., 29:* 74-9.
9. Bucke, C., D. A. Walker and C. W. Baldry. 1966. Some effects of sugar and sugar phosphates on carbon dioxide fixation by isolated chloroplasts. *Biochem. J., 101:* 636-41.
10. Coombs, J. and C. P. Whittingham. 1966. The mechanism of inhibition of photosynthesis by high partial pressures of oxygen in *Chlorella*. *Proc. Roy. Soc. Lond., 164B:* 511-20.
11. Ellyard, P. W. 1968. The Warburg effect, investigations using isolated spinach chloroplasts. Thesis. Cornell University, Ithaca, New York.
12. Fewson, C. A., C. C. Black and M. Gibbs. 1963. Further studies on the photochemical production of reduced triphosphopyridine nucleotide and adenosine triphosphate by fragmented spinach chloroplasts. *Plant Physiol., 38:* 680-85.
13. Forrester, M. L., G. Krotkov and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. II. Corn and other monocotyledons. *Plant Physiol., 41:* 428-31.
14. Hatch, M. D. and J. F. Turner. 1960. The mechanism of the reversible aerobic inhibition of glycolysis in a pea seed extract. *Biochem. J., 75:* 66-72.
15. Heber, U. W. and K. A. Santarius. 1965. Compartmentation and reduction of pyridine nucleotides in relation to photosynthesis. *Biochim. Biophys. Acta, 109:* 390-408.
16. Hess, J. L. and N. E. Tolbert. 1967. Glycolate pathway in algae. *Plant Physiol., 42:* 371-79.
17. Keister, D. L., A. San Pietro and F. E. Stolzenbach. 1960. Pyridine nucleotide transhydrogenase from spinach. *J. Biol. Chem., 235:* 2989-96.
18. Latzko, E. and M. Gibbs. 1968. Effect of O_2, arsenite and sulfhydryl compounds and light on the activity of ribulose 5-phosphate kinase. International Congress of Photosynthesis, Freudenstadt *44:* 396-402.

19. Latzko, E. and M. Gibbs. 1969. Level of photosynthetic intermediates in isolated spinach chloroplasts. *Plant Physiol.* (in press).
20. McAlister, E. D. and J. Myers. 1940. The time course of photosynthesis and fluorescence observed simultaneously. *Smithsonian Misc. Coll., 99:* No. 6.
21. Miyachi, S., S. Izawa and H. Tamiya. 1955. Effect of oxygen on the capacity of carbon dioxide fixation by green algae. *J. Biochem., 42:* 221-4.
22. Miyachi, S., S. Hirokawa and H. Tamiya. 1955. Effect of quinone on the light-induced capacity of carbon dioxide fixation in green algae. *J. Biochem., 42:* 737-43.
23. Ozbun, J. L., R. J. Volk and W. A. Jackson. 1967. Effects of light and darkness on gaseous exchange of bean leaves. *Plant Physiol., 39:* 523-27.
24. Tamiya, H. and H. Huzisige. 1949. Effect of oxygen on the dark reaction of photosynthesis. Stud. Tokugawa Inst., *6:* 83-104.
25. Tanner, H. A., T. E. Brown, C. Eyster and R. W. Treharne. 1960. A manganese dependent photosynthetic process. *Biochem. Biophys. Res. Comm., 3:* 205-10.
26. Turner, J. S., M. Todd and E. G. Brittain. 1956. The inhibition of photosynthesis by oxygen. I. Comparative physiology of the effect. *Aust. J. Biol. Sci., 9:* 494-510.
27. Turner, J. S. and E. G. Brittain. 1962. Oxygen as a factor in photosynthesis. *Biol. Rev., 37:* 130-70.
28. Turner, J. S., J. F. Turner, K. D. Shortman and J. E. King. 1958. The inhibition of photosynthesis by oxygen. II. The effect of oxygen on glyceraldehyde phosphate dehydrogenases from chloroplasts. *Aust. J. Biol. Sci., 11:* 336-42.
29. Warburg, O. 1920. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. *Biochem. Z., 103:* 188-217.
30. Warburg, O. and G. Krippahl. 1960. Glykolsäurebildung in *Chlorella*. *Z. Naturforsch., 15b:* 197-99.
31. Zelitch, I. 1964. Organic acids and respiration in photosynthetic tissues. *Ann. Rev. Plant Physiol., 15:* 121-42.
32. Zelitch, I. 1965. The relation of glycolic acid synthesis to the primary photosynthetic carboxylation reaction in leaves. *J. Biol. Chem., 240:* 1869-75.

How Crops Are Modified

O. E. Nelson, Jr.

In expanding on a topic introduced in *How Crops Grow,* I've chosen to discuss how crops are modified to suit better man's need. I do not intend to list the changes effected in wild species during their association with man nor the methods by which these changes can be wrought. Rather I wish to illustrate the thesis that as our knowledge of living systems increases so also does our ability to manipulate the systems for our ends.

The modification of plants to be more useful to man is a process that has been going on continually since prehistoric tribes of man changed from a food gathering to an agricultural mode of life in certain favorable locations. The modification was one in which many patient and obviously perceptive husbandmen joined. Remarkable progress was made before the discovery of the scientific foundation for the improvement of plants: Mendel's laws. All plants we use today had become cultivated plants, and man had learned to exploit the variability present and to select towards desired ends. One of the most striking examples of the evolution of cultivated plants is the group of cultivated plants descended from the wild *Brassica oleracea* of western Europe by the selection of desirable variants.

In the fourth century B.C. the Greeks cultivated both smooth and curly leaf cabbages. The ancient Romans had these leaf cabbages and a form of broccoli. By the time of Charlemagne in the 8th century, kohlrabi was being cultivated. Head cabbages made their appearance in the 12th century, and by the 16th century cauliflower was being grown in Italy. Brussel sprouts made their appearance in Belgium at about the end of the 18th century (Schwanitz, 1967).

Charles Darwin knew that the twin keys to the modification of domestic plants or domestic animals were variation and selection. In his epochal "Origin of Species" (1859) he used extensively the variations noted in domesticated plants and animals. In fact, the first chapter in the "Origin of Species" is entitled "Variation Under Domestication." In this chapter, Darwin notes:

> When we compare the host of agricultural, culinary, orchard, and flower garden races of plants, most useful to man at different seasons

> and for different purposes, or so beautiful in his eyes, we must, I think, look further than to mere variability. We cannot suppose that all the breeds were suddenly produced as perfect and as useful as we now see them; indeed, in several cases, we know that this has not been their history. The key is man's power of accumulative selection: nature gives successive variations; man adds them up in certain directions useful to him. In this sense he may be said to make for himself useful breeds.

In spite of substantial achievements in the realm of plant breeding, the rediscovery of Mendel's laws at the beginning of this century and the development of genetics as a science gave new tools to the plant breeders. They now had a scientific foundation for what had previously been an art. In the course of genetic investigations, geneticists learned to exploit the phenomenon of hybrid vigor. They learned how to deal most effectively with selection for characters that are conditioned by a number of genes. They acquired the ability to generate variability experimentally and at will. In some instances, they even learned to transfer genes from one genus to another (Sears, 1956). Together with biochemists and plant physiologists, they have acquired increasing knowledge of the basic aspects of higher plants that may enable them to modify crops to man's ends more drastically than was once judged possible. It is almost a truism to state that the better we understand the metabolism of plant systems, the better we are able to understand what variations are admissible and to turn these to our own ends.

CEREAL PROTEINS

Today I should like to illustrate this point by reference to investigations carried on at Purdue with my colleague, Professor Edwin Mertz, with the view to increasing the protein value of maize. This strikes me as a particularly appropriate topic for this audience and at this time because the solution has its conceptual basis in pioneering research carried on here at the Station by T. B. Osborne in the early years of this century. The world shortage of good quality protein is a subject of much interest. Protein malnutrition is now a real problem and may become even more so in the very near future (President's Science Advisory Committee, 1967). The possibilities of improving the protein quality of cereal grains have long been explored but until recently without success. The proteins of all cereal grains are deficient in the essential amino acid, lysine. In addition to a deficiency of lysine, maize protein is also deficient in another essential amino acid, tryptophane.

When in 1963 Professor Mertz and I began discussing the problem of improving maize protein quality, we had two advantages over our predecessors. In the first place, at that time, the extent of gene control of protein synthesis was clear. Since the sequence of the amino acids within every protein synthesized in an organism is coded for by the hereditary material, it was already clear that there are only special instances in which one could expect to induce large changes in the

amino acid composition of the composite proteins. Our second advantage was an automatic amino acid analyzer.

MAIZE SEED PROTEIN FRACTIONS

Normal. To understand our approach to the problem, one must know something of the distribution of the protein fractions in the maize seed. About 16 per cent of the protein in the seed is in the embryo and associated scutellum. The composite proteins of the embryo are much superior in nutritive value to those of the endosperm. The endosperm contains about 81 per cent of the protein of the seed. The remaining 3 per cent is accounted for by the seed coat. The proteins of the cereal grains were early investigated at this Station by T. B. Osborne and his colleagues (Osborne, 1924). They fractionated proteins on the basis of their solubility. The albumin fraction was soluble in distilled water, globulin in dilute sodium chloride, prolamine in 70 per cent ethanol, and most of the remaining protein (the glutelins) in dilute sodium hydroxide. A minor portion of the protein is not extracted by any of the solvents employed.

For example, we find, using a modified Osborne method of extraction, approximately 4 per cent of the endosperm proteins can be extracted by water, 2 per cent by sodium chloride, 54 per cent by ethanol, and 40 per cent by the dilute sodium hydroxide solution. These percentages may vary somewhat from one variety or hybrid to another. In extracts of the entire seed there are higher percentages of protein extractable by water and dilute salt solution because almost all the embryo protein is extractable in these solvents. The large ethanol-soluble fraction is our chief concern. Osborne and Mendel (1914) found no detectable lysine or tryptophane in this fraction. They reported that rats of all ages went into a rapid decline and ultimately died when placed on a diet in which the sole source of protein was the ethanol-soluble protein (zein) from maize.

Therefore, more than half of the endosperm protein and almost half of the entire seed protein in maize is of a type that could be characterized as being nutritionally worthless since it contributes no tryptophane and no lysine (or very little). This high proportion of alcohol-soluble protein is typical of all the cereals where lysine is the limiting amino acid (Mossé, 1966).

Mutants. We could then state the problem: can mutants be found that block the synthesis of the ethanol-soluble fraction and enhance the other fractions which are more valuable for nutrition? In this phase of the investigation we were remarkably fortunate. I had thought that if such mutants had been isolated they might well be the mutants known as "opaque" or "floury." Accordingly, I supplied Professor Mertz with seeds of the *opaque-1*, *opaque-2*, *floury-1* and *floury-2* mutants. Analyses soon indicated that both *opaque-2* and *floury-2* had lysine contents substantially above normal maize or those of the *opaque-1* and *floury-1*

mutants (Mertz *et al.*, 1964 and Nelson *et al.*, 1965). All of these mutants had been isolated a number of years previously because of their phenotypic effect on the appearance of the seed. Although one group of mutants is called opaque and the other floury, phenotypically they both have a dull opaque appearance and are indistinguishable one from the other. The opaque appearance of these mutant kernels is due to the absence of the corneous starch found in abundance in the endosperm of normal maize seeds (Figs. 1 and 2). The mutants are classified as opaque or floury by their mode of inheritance. The *opaque-1* and *opaque-2* mutations are simple Mendelian recessives whereas *floury-1* and *floury-2* are semi-dominant genes.

In using the *opaque-2* and *floury-2* mutations in plant breeding, the opaque phenotype of the seed is a distinct advantage. One can follow the transfer of the gene into the desired background simply by the phenotypes of the seeds. Chemical analyses at every step in a breeding program are unnecessary.

The *opaque-2* mutant was isolated at The Connecticut Agricultural Experiment Station in the late 1920's by Drs. Jones and Singleton. The mutant, *floury-2*, was isolated by Dr. Mumm at the University of Illinois at about the same time (Emerson *et al.*, 1935).

The *opaque-2* and *floury-2* mutations lead to useful increases in the concentration of not only lysine but also the other limiting amino acid in maize protein, tryptophane. Table 1 shows the amino acid content of the composite endosperm proteins of normal maize, *opaque-2* maize, and *floury-2* maize. Only those amino acids are listed in which a substantial change in concentration is found in the mutants as compared to normal. At the top are the amino acids in which there is an increase in one or both mutants as compared to normal maize. Note that the

Fig. 1. A median transverse section of a mature kernel of normal maize with the embryo at the upper center. The corneous starch extends almost completely around the periphery of the endosperm.

Fig. 2. A median transverse section of a mature kernel of *opaque-2* maize with the embryo at the upper center. No corneous starch is present in the endosperm.

Table 1. Amino acid concentrations (gms per 100 gms protein) in endosperm proteins of normal, opaque-2, and floury-2 maize

	+*	o_2*	fl_2
Lysine	1.6	3.7	3.3
Tryptophan	0.3	0.7	0.8
Histidine	2.9	3.2	2.6
Arginine	3.4	5.2	4.5
Aspartic	7.0	10.8	9.2
Glycine	3.0	4.7	3.4
Glutamic	26.0	19.8	19.1
Alanine	10.1	7.2	8.0
Leucine	18.8	11.6	13.3
Phenylalanine	6.5	4.9	5.1
Tyrosine	5.3	3.9	4.5
Cystine	1.8	[ ]	1.8
Methionine	2.0	1.8	3.2

* Normal and *opaque-2* compared in W64A background.

levels of lysine and tryptophane are nearly double in the mutants compared to normal. In the middle are listed the amino acids in which one finds a decrease in the mutants as compared to normal. The lower part of the table gives the values for the sulphur-containing amino acids. There is little change in *opaque-2* as compared to normal with regard to the concentration of cysteine and methionine. The missing value in the table for cysteine under *opaque-2* does not indicate our inability to find this amino acid in *opaque-2,* but simply that this was not a good analysis. Characteristically, the levels of cysteine in *opaque-2* are slightly higher than in normal. The concentration of methionine in the *floury-2* mutant is greater than in either normal or *opaque-2*. This is particularly interesting since in mixtures of corn and legumes, often employed in some sections of Latin America for human food, methionine tends to be the first limiting amino acid.

Table 2 gives the amino acid composition of embryos from normal and *opaque-2* maize. The very pronounced differences between genotypes for certain amino acids characteristic of the endosperm proteins are not found in the embryo proteins. The minor differences between the two genotypes are due to variability in the analysis. The amino acid composition of the composite embryo proteins is quite different from that of the endosperm proteins. The embryo proteins are better balanced, from the standpoint of providing a protein source for humans or monogastric animals, than the endosperm proteins.

There are no differences in amino acid pattern between *opaque-2* and normal leaves, or between *opaque-2* and normal pollen (Nelson,

Table 2. Amino acid composition (gms per 100 gms protein) of defatted maize embryos [5]

Amino acid	Normal	*Opaque-2*
Lysine	6.1	5.9
Histidine	2.9	2.9
Ammonia	2.2	2.1
Arginine	9.1	9.2
Aspartic acid	8.2	9.2
Glutamic acid	13.1	13.9
Threonine	3.9	3.7
Serine	5.5	5.0
Proline	4.8	5.3
Glycine	5.4	5.5
Alanine	6.0	5.8
Valine	5.3	4.4
Cystine	1.0	0.9
Methionine	1.7	1.5
Isoleucine	3.1	2.5
Leucine	6.5	5.6
Tyrosine	2.9	2.2
Phenylalanine	4.1	3.6

1968). Therefore, the altered amino acid composition characteristic of *opaque-2* endosperm proteins apparently is confined to that tissue.

Table 3 gives the amino acid concentrations of hydrolysates of entire seeds for the three genotypes. In our nutritional investigations (see below) we always used the entire seed rather than the endosperm alone. Most of the changes characteristic of the endosperm proteins can be noted in the collected proteins of the whole seed, but their magnitude is smaller. The increased nutritional advantage of *opaque-2* and *floury-2* as compared to normal depends largely on the increased quantities of lysine and tryptophane. Cromwell *et al.* (1967) showed that supplementing normal maize with lysine and tryptophane to the levels found in *opaque-2* will support the growth of weanling pigs at a rate nearly equal to that found with *opaque-2* maize. One other change of possible consequence is the lower amount of leucine in the mutant proteins. Since high quantities of leucine are capable of antagonizing the use of isoleucine, and possibly also of tryptophane, the lower quantity of leucine in *opaque-2* maize may be beneficial.

We were particularly interested in understanding how the changes in amino acid composition arise because of the relationship between the genetic material and the amino acid sequence of proteins. It has also been of interest to us as a test of the hypothesis with which we originally embarked on this search. Jorge Jiménez, one of my graduate students, investigated the endosperm proteins synthesized by the different genotypes of maize (Jiménez, 1968). Table 4 gives the amount of protein extractable from 10 grams of defatted endosperm and from a single

Table 3. Amino acid composition (gms per 100 gms protein) for defatted whole kernels of opaque-2, floury-2, and normal maize. 1967 crop

Amino acid	*o*$_2$	+	*fl*$_2$
Lysine	5.0	3.0	4.8
Tryptophan	1.3	0.7	....[a]
Histidine	3.5	2.6	2.9
Arginine	7.2	4.9	6.3
Aspartic acid	8.8	9.2	10.5
Threonine	3.8	4.1	4.1
Serine	4.7	5.6	5.2
Glutamic acid	17.2	22.6	18.5
Proline	8.4	9.6	8.8
Glycine	5.1	4.7	4.7
Alanine	6.7	9.2	8.0
Cystine	2.0	1.7	1.6
Valine	5.2	5.7	5.7
Methionine	2.2	1.3[b]	2.7
Isoleucine	3.4	4.2	4.0
Leucine	9.3	14.6	12.0
Tyrosine	4.2	5.2	4.6
Phenylalanine	4.4	5.8	5.2
% Protein	10.5	9.0	17.0

[a] No analysis available. Previous results show tryptophan values for *floury-2* equal to *opaque-2*.

[b] Lower than expected. Some may have been destroyed during hydrolysis.

Table 4. Protein fractions extracted from defatted endosperms of various genotypes. Data of J. Jiménez

		+		*o*$_2$		*fl*$_2$	
		A[a]	B[b]	A	B	A	B
H_2O	mg	53.8	0.92	152.5	1.92	123.3	1.26
	%[c]	3.8		12.1		9.6	
5% NaCl	mg	28.2	0.48	64.3	0.81	92.6	0.95
	%	2.0		5.1		7.3	
70% ETOH	mg	775.2	13.34	288.5	3.64	370.7	3.80
	%	55.1		22.9		29.0	
0.2% NaOH	mg	447.6	7.70	629.0	7.93	521.3	5.35
	%	31.8		50.1		40.8	
Residue	mg	47.9	0.82	55.6	0.71	182.9	1.88
	%	3.4		4.5		14.9	

[a] per 10 gm

[b] per endosperm

[c] of total protein

endosperm by the various solvents. Note the great reduction in the amount of alcohol-soluble proteins synthesized by the two mutants as compared to normal. This is coupled with an increased quantity of water-soluble and salt-soluble proteins in the mutants. There is, however, no increase in the amount of alkali-soluble protein present in the mutants when the data are expressed on a per endosperm basis. The synthesis of the ethanol-soluble fraction is not completely blocked in the two mutants, but it is very substantially reduced. Dr. Jiménez has also obtained the amino acid composition of the various solubility fractions shown in Table 4. The water-soluble, salt-soluble, and ethanol-soluble fractions show no changes in amino acid concentration expressed as grams per 100 grams of protein. There is, however, a detectable change in the alkali-soluble fraction. The fraction derived from *opaque-2* has more lysine and more arginine, but somewhat less glutamate and proline than is characteristic of normal maize. The same types of changes seem to be characteristic of *floury-2* also (Table 5).

Different amino acid compositions of the collected proteins could arise in several different ways. Some new species of proteins could be synthesized. Alternatively changes in the proportions of proteins normally synthesized could account for differences in amino acid composition without any new proteins being synthesized. Jiménez has studied the electrophoretic mobility of the isolated proteins in starch-gel-urea electrophoresis. The water-soluble and salt-soluble fractions of normal and the mutants show no qualitative differences. The ethanol-soluble fractions of the two mutants do show qualitative differences from normal as illustrated in Figure 3. For *opaque-2* 3 proteins observed in normal are missing but the proteins that are present correspond to those in normal on the basis of electrophoretic mobility. The ethanol-soluble fraction of *floury-2* has as many proteins as the same fraction from normal. However, one protein is altered in its mobility and moves slightly more rapidly. The alkali-soluble fractions of *opaque-2* and normal are qualitatively similar (Figure 4). There are, however, quantitative differences between them. Several bands appear to be reduced in intensity in the *opaque-2* mutant. A band close to the origin in normal and *opaque-2* is missing from the *floury-2* mutant.

We conclude that no new or different proteins are synthesized by the *opaque-2* mutant. Three proteins in the alcohol-soluble fraction are

Table 5. Amino acids in the alkali-soluble fraction (insoluble at pH 6.0) of +, o_2, and fl_2 endosperms. Expressed as gms per 100 gms protein. Data of J. Jiménez

Amino acid	+	o_2	fl_2
Lysine	3.2	4.5	4.4
Arginine	4.8	5.7	4.9
Glutamate	19.8	18.2	18.1
Proline	7.7	5.9	7.3

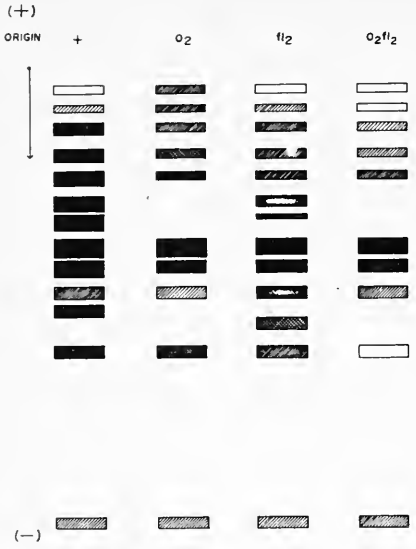


Fig. 3. The separation of the alcohol-soluble proteins of +/+/+, $o_2/o_2/o_2$, $fl_2/fl_2/fl_2$, and $o_2/o_2/o_2$ $fl_2/fl_2/fl_2$ endosperms by starch gel electrophoresis with aluminum lactate-lactic acid buffer, pH 3.5. Duration of the run was 22 hours at 2.2 mA/gel. The stain was light yellow green SF.

not produced in the mutant. In the *floury-2* mutant one protein is altered in its electrophoretic mobility but the overall effect of the mutation is similar to that of *opaque-2*. We think that the changes in amino acid composition are a consequence of changes in the proportions of the various solubility fractions of different amino acid composition that are synthesized in the two mutants.

The avenues leading to increased protein quality in plants are limited. The genetic information which specifies the amino acid sequence of every protein synthesized by an organism tends to preserve a given amino acid composition for each protein. A mutational change in amino acid sequence may make a protein non-functional. If the protein is essential for growth or development of the organism, such a mutation is lethal. For this reason it is rather unlikely that the amino acid composition of leaf proteins could be substantially altered since most of the

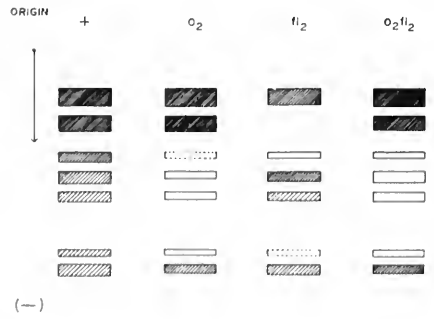


Fig. 4. The separation of the alkali-soluble proteins precipitable at pH 6 of +/+/+, $o_2/o_2/o_2$, $fl_2//fl_2/fl_2$, and $o_2/o_2/o_2$ $fl_2/fl_2/fl_2$ endosperms by starch gel urea electrophoresis with aluminum lactate-lactic acid buffer, pH 3.5. Duration of the run was 12 hours at 2.2 mA/gel. Nigrosine was used as a stain.

protein present is associated with chloroplasts and is undoubtedly functional in a metabolic or a structural sense.

While changes in the translation mechanism could conceivably have marked effects on the relative concentrations of two amino acids it is unlikely that this is so. A given transfer RNA (e.g., for amino acid A) could be changed by mutation so that it could accept another amino acid (B) and subsequently insert it in a number of positions where A is specified in all the proteins being synthesized. It seems unlikely that this could be an effective means of changing the amino acid composition of the proteins being synthesized, even if it were not lethal, because of the genetic redundancy of transfer RNAs. If most higher organisms have a degree of redundancy of transfer RNA typical of that found by Ritossa *et al.* (1966) for *Drosophila,* then a change in one particular transfer RNA gene would have a negligible effect upon the overall amino acid composition of the proteins being synthesized.

It appears at the present time that the improvement of protein quality in cereals is limited to a change in the proportion of alcohol-soluble proteins relative to the other proteins being synthesized or a change in the relative proportion of the endosperm proteins to the embryo proteins which have a more desirable amino acid composition. I have discussed the rationale underlying this conclusion in greater detail elsewhere (Nelson, 1969).

Feeding studies. With the realization that the *opaque-2* mutant contains appreciably higher concentrations of lysine and tryptophane than common corn, we were most eager to undertake nutritional studies to confirm that these higher concentrations of the limiting amino acids were biologically available. In 1964, Miss Veron undertook the first nutritional trials using weanling male rats. Figure 5 shows two rats taken from those on trial. The rat on the left was fed for 4 weeks on a diet of 90% *opaque-2* maize, 5% corn oil, 4% mineral mix and 1% vitamin mix. The rat on the right was fed over the same period, a similar diet with common maize substituted for *opaque-2* maize. During the test period, the rats fed on *opaque-2* maize gained weight 3½ times as rapidly as the rats fed on common maize. The increased concentration of lysine and tryptophane was reflected in the greater growth of the test animals (Mertz *et al.,* 1965).

In 1965, we produced sufficient *opaque-2* maize for large scale trials with weanling pigs (Cromwell *et al.,* 1967). Figure 6 shows two pigs taken at random from a later trial testing the nutritional quality of *opaque-2* and common maize for swine. Both pigs went on test with an initial weight of 23 lbs. After being fed on corn as a sole source of energy and proteins with a supplement of minerals and vitamins for 42 days, the pig fed on common corn had gained 7½ lbs. The pig fed on *opaque-2* maize had gained 34 lbs. Such results have confirmed the greater nutritional quality of *opaque-2* maize for monogastric animals.

Most gratifyingly, *opaque-2* maize appears to have much greater nu-

Fig. 5. Rats representative of those fed on either *opaque-2* maize (left) or common maize (right) as a sole source of protein for 4 weeks after weaning in the first test of the nutritional quality of *opaque-2* maize.

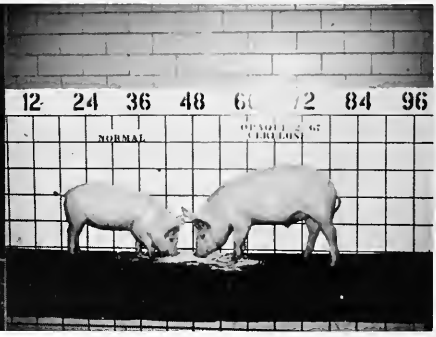


Fig. 6. Pigs fed for 42 days after weaning on *opaque-2* maize (right) or common maize (left) as a sole source of protein. Cerelose was added to the *opaque-2* maize diet to make the diets isonitrogenous.

tritional value than common maize for children. Part of the 1965 crop of *opaque-2* corn was sent to Ricardo Bressani at the Institute of Nutrition of Central America and Panama. The data given here are taken from Bressani (1966). In working with children, it is not possible to do the same type of experiment as is done with experimental animals. Instead, the nutritional quality of common and *opaque-2* corn was tested by measuring the input of dietary nitrogen and nitrogen excretion. The fecal nitrogen is a measure of the amount of protein not absorbed in passage through the intestinal tract. The urinary nitrogen measures the nitrogen derived from metabolic processes. The amount of nitrogen that is absorbed minus the urinary nitrogen gives a measure of the nitrogen being retained by the body. The better the nutritional quality of the protein source, the higher the percentage of protein that is retained by a growing child if proper levels of nitrogen input are selected. The strategy in Bressani's experiments was to equilibrate the experimental subjects on skimmed milk as a protein source for 4 days and then run a test of 4 days in which the nitrogen input and output was precisely measured when all the protein in the children's diet came from skimmed milk. This was followed by an equilibration period for 4 days with corn as a source of protein, and finally the nitrogen balance of children on diets in which all the protein came from maize was measured in a subsequent 4 day period. Table 6 presents the results of an experiment in which Bressani compared the protein quality of skimmed milk and common corn. Note that when the children were fed on common corn they excreted more nitrogen than they consumed. This is a certain indication of the poor quality of the corn protein, since the children were in negative nitrogen balance. In Table 7 we see a similar test done with 4 children fed skimmed milk or *opaque-2* corn as

Table 6. Nitrogen balance of children fed skim milk and common Guatemalan corn masa. (Bressani, 1966)

	Milk	Corn masa
Nitrogen (mg N/kg/day)		
Intake	315	319
Fecal	54	65
Urine	199	268
Absorbed	261	254
Retained	62	−14
% N Absorption	82.9	79.6
% N Retention	19.7	0

sole protein sources in alternating test periods. The critical datum is the amount of nitrogen retained, and in this test the values were of a similar magnitude for *opaque-2* corn and for skimmed milk. This is an indication that at these levels of nitrogen intake *opaque-2* corn protein is approximately equal in quality to skimmed milk protein.

Other experiments with children were carried out in Cali, Colombia by Harpstead *et al.* (1968) and Pradilla *et al.* (1968) who found that children can recover from the severe protein deficiency syndrome known as kwashiorkor on a diet in which all the amino acids are supplied by *opaque-2* maize. They were able to reduce the input of *opaque-2* maize to 75% of the nitrogen consumed and supply the balance as non-essential nitrogen in the form of glycine or diammonium citrate. Under such circumstances they found that the children are still capable of quickly assuming a positive nitrogen balance with a subsequent recovery of normal health.

Table 7. Nitrogen balance of four children fed skim milk and opaque-2 corn masa. (Bressani, 1966)

	Milk	*Opaque-2* Corn	Milk
Nitrogen (mg N/kg/day)			
Intake	274±15	300±5	271±10
Fecal	49±25	71±11	42±22
Urine	159±22	142±18	153±28
Absorbed	225±19	229±13	229±21
Retained	66±14	87±19	76±19
% N Absorption	82.1	76.3	84.5
% N Retention	24.1	29.0	28.0
Ave. Wt. Kg.	12.56	13.13	13.38

The results of all feeding experiments to date with *opaque-2* maize on humans and monogastric animals have been similarly encouraging.

CONCLUSIONS

Protein deficiency is an important aspect of the world's food problem (President's Science Advisory Committee, 1967). Cereals supply about 100 million tons of protein per year, and a large proportion of this is consumed directly by humans. By contrast, legumes add about 20 million tons of protein per year and animal protein another 20 million tons of protein to the world's supply (Altschul, 1965). If the cereals had better amino acid balance and hence better nutritional quality than is currently true, we would make large strides towards meeting the protein deficiency of the world.

As already noted, all the cereals are notably deficient in lysine. In addition to lysine, maize is also deficient in tryptophane. For all the other cereals, threonine appears to be the second limiting amino acid (Howe *et al.*, 1965). There are various means of rectifying the deficiency of the essential amino acids in cereal grains. Hopefully, mutations that enhance the concentration of lysine can be identified in cereals other than maize. Flour can be supplemented with synthetic amino acids; leguminous meals can be mixed with cereal flours in order to achieve a better balance than is found in the cereals alone; inexpensive protein concentrates such as those from fish, leaf proteins or microorganisms can be added to cereal flours. These possibilities are not mutually exclusive alternatives. If the projections for population growth and food production over the next 30 years are correct (Bonner, 1967), we will need and must employ every strategy for obtaining nutritionally adequate proteins.

I would like to point out, however, that the integral improvement of traditional foods is certainly most desirable where feasible. There is a relatively small problem in introducing a new strain and inducing the population to use it. The distribution problems encountered with synthetic amino acid or protein concentrate supplement are avoided. I would predict that mutations like *opaque-2* exist in any cereal that synthesizes large quantities of alcohol-soluble proteins such as wheat, rye, barley, millet and sorghum (Mossé, 1966).

In conclusion, I should like to return to my primary thesis, that the more we learn basically about the systems we use, the better equipped we are to modify them to our own ends. Charles Darwin in the first chapter of "The Origin of Species" noted that "Man can hardly select, or only with great difficulty, any deviation of structure excepting such as is externally visible; and indeed he rarely cares for what is internal." I suggest that today this need apply only where the geneticists and the plant breeders are unwilling to utilize our existing knowledge.

December 16, 1968

LITERATURE CITED

Altschul, Aaron (1965) "Proteins—Their Chemistry and Politics." Basic Books, New York.

Bonner, James (1967) In "The Next Ninety Years." Proc. of a Conference at Calif. Inst. of Tech. Ed., R. P. Schuster. Calif. Inst. of Tech., Pasadena.

Bressani, Ricardo (1966) Protein quality of *opaque-2* maize in children. In "Proc. High Lysine Corn Conf., Purdue University." (E. T. Mertz and O. E. Nelson, eds.) Corn Industries Research Found., Wash., D.C.

Cromwell, G. L., R. A. Pickett, and W. M. Beeson (1967) Nutritional value of *opaque-2* corn for swine. J. An. Sci. 26: 1325.

Darwin, Charles (1859) "On the Origin of Species." John Murray, London.

Emerson, R. A., G. W. Beadle, and A. C. Fraser (1935) A summary of linkage studies in maize. Cornell Univ. Agr. Expt. Sta. Mem. 180.

Harpstead, D. D., A. Pradilla, and D. Sarria (1968) *Opaque-2* maize as a protein source for the treatment of malnutrition in humans. Agron. Abstr. 66.

Howe, E. E., G. R. Jansen, and E. W. Gilfillan (1965) Amino acid supplementation of cereal grains as related to the world food supply. Amer. J. Clin. Nutrit. *16:* 315.

Jiménez, J. R. (1968) The effect of the *opaque-2* and *floury-2* genes on the production of protein in the maize endosperm. Ph.D. Thesis, Purdue Univ.

Mertz, E. T., O. E. Nelson, and L. S. Bates (1964) Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science *145:* 279.

Mertz, E. T., O. A. Veron, and O. E. Nelson (1965) Growth of rats fed on *opaque-2* maize. Science *148:* 1741.

Mossé, J. (1966) Alcohol-soluble proteins of cereal grains. Fed. Proc. *25:* 1663.

Nelson, O. E. (1969) Genetic modification of protein quality in plants. Adv. in Agron. (In press.)

Nelson, O. E. (1968) The modification by mutation of protein quality in maize. In "Breeding for Improved Plant Protein." Proceedings of an IAEA-FAO Panel. Röstånga, Sweden, 17-21 June.

Nelson, O. E., E. T. Mertz, and L. S. Bates (1965) Second mutant gene affecting maize endosperm proteins. Science *150:* 1469.

Osborne, T. B. (1924) "The Vegetable Proteins." Longmans, Green and Co., London.

Osborne, T. B. and L. B. Mendel (1914) Amino acids in nutrition and growth. J. Biol. Chem. *17:* 325.

Pradilla, A., F. Linares, and D. D. Harpstead (1968) Plant protein mixtures based on high lysine maize. Agron. Abstr. 68.

President's Science Advisory Committee (1967) Report of Panel on the World Food Supply. Vols. I and II. U. S. Gov't. Printing Office, Washington, D.C.

Ritossa, F. M., K. C. Atwood and S. Spiegelman (1966) On the redundancy of DNA complementary to amino acid transfer RNA and its absence from the nucleolar organizer region of *Drosophila melanogaster.* Genetics *54:* 663.

Schwanitz, F. (1967) "The Origin of Cultivated Plants." Harvard Univ. Press, Cambridge, Mass.

Sears, E. R. (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp. Biol. *9:* 1.

How Plants Get and Use Nutrients

Sterling B. Hendricks

Samuel Johnson in writing a century ago on *How Crops Grow* (1) was concerned not only with the basic question but also with the practical matter of how to grow crops. Theory and practice change with time and a century of work has led to advances. Although many aspects of plant nutrition are not yet understood the use of fertilizers to meet nutrient requirements has been very effective. Johnson would surely have appreciated this.

I will first say something about fertilizers and the supply of nutrients from soils before turning to the "How" of plant nutrient uptake.

The recognition of a problem is of prime importance: it stirs the imagination even though immediate understanding may not result. Samuel Johnson's time was one of recognition of the needs of plants for fertilizer nutrients. Plant nutrition had the aura of excitement that nucleic acids have today. One could begin to sense the connections between plant life and three of the four elements of the Greeks: light, earth, and air.

Johnson's contemporaries included Liebig, Boussingault, Lawes, and Gilbert who established the plant's needs for nutrients from the earth and sought ways to meet these needs. Analyses of plant ash, the basis for both Liebig's and Johnson's approach to nutrition, show what elements are absorbed in plant growth. Values in Table 1 are taken from *How Crops Grow* and are comparable with those in current literature. They indicate a need for large amounts of potassium, calcium, mag-

Table 1. Composition of ash from turnip tops (1)

Component	Per cent	Component	Per cent
K_2O	28.1	Fe_2O_3	0.8
CaO	34.8	P_2O_5	7.6
MgO	2.5	SO_3	6.5
Na_2O	6.0	Cl	4.7
Total ash 10.9% of dry weight			

nesium, phosphorus, and sulfur. Johnson appreciated from the analyses of many plants that sodium was not universally present and thus probably not essential. It is interesting that he considered sodium as capable of partially replacing potassium as a nutrient for field beets, a point still being studied. While he recognized iron as essential he thought manganese non-essential. He also appreciated the importance of nitrogen and was aware of the work of Boussingault on including legumes in crop rotations.

Supply and Use of Fertilizers

The American farmer in the 19th century did not have the animal manures of the Old World described by Fitzherbert in 1523 in "The Boke of Husbandry." He was plagued by infertile soils: land was "worn out" even in Washington's time. It was Johnson's contemporary, Edwin Ruffin, who first realized the importance of liming. The Virginia planter described the need for calcium in "An Essay on Calcareous Manures," published in 1832 (2). Bone meal as a source of phosphorus was first used in about 1850 when some 5000 tons were sold in the U. S., accompanied by a discussion of the merit of treating it with sulfuric acid. Guano, which provides both nitrogen and phosphorus, was first used in 1824 by John Stuart Skinner the editor of "The American Farmer." Imports of guano reached a maximum of 175,000 tons in 1854 but declined in the Civil War. Commercial manufacture of superphosphate started in the U.S. in 1852 following the work of Gilbert and Lawes at Rothamsted. The fertilizer firm of Baugh and Sons, which is still in business in Baltimore, started superphosphate production in 1855. Potassium carbonate in wood ashes, or potash, completed the supply of the major nutrient elements recognized by Johnson as essential for plant growth.

An adequate base for supply of the common fertilizer elements, together with ground limestone, had formed by 1900. It depended on superphosphate made from South Carolina, Florida, and Tennessee phosphate rock treated with sulfuric acid from several sources. Potassium chloride was imported from the extensive deposits in Stassfurt, Germany. Sodium nitrate came from the deserts of northern Chile to augment ammonia, a by-product of the coke industry. The native deposits of phosphate rock thus made the United States self-sufficient in fertilizer phosphorus. Similarly, the sulfur found associated with salt domes in Texas, coupled with the ingenious Frasch steam-mining process, made our supply of this element complete. More than 26 million tons of phosphate rock, and an equal amount of limestone, are now used each year in American agriculture.

The problem of potash supply was also solved by discoveries of American sources. The first, discovered in the 1920's, were the Searle Lake deposits in California, followed by mines in the Carlsbad region of New Mexico. Current resources in eastern Utah and Saskatchewan

are adequate to meet needs for the foreseeable future. About 3 million tons of K_2O are now used each year in agriculture.

The supply of native plant nutrients to American farms was perfected in the 20th century. The greatest need was a synthetic source of nitrogen compounds. This was fulfilled by the Haber-Bosch process for hydrogenation of nitrogen to form ammonia. Although used in Germany by 1914, prolonging World War I by at least a year, the process did not come into use in the U. S. until 1925. The exploitation of petroleum sources has afforded ample supplies of hydrogen so that the price of ammonia has steadily fallen. More than 5 million tons of NH_3 per year are now used in agriculture or enough for 200 million acres at a rate of 50 lbs NH_3 per acre.

I will illustrate the progress in agricultural production from fertilizer use by the yield of maize in the U. S. between 1933 and 1963 (Table 2). Production over this period increased by two-thirds although the acreage was reduced by 43%. This represents a threefold increase in yield per acre. The first surge in productivity came from mechanization and use of hybrids—the second from increased fertilizer use. Mechanization, plant breeding, fertilizer supply, and agricultural chemicals are four supporting legs on which agriculture now depends.

Our plant nutrient supplies and land reserve are adequate to meet the needs for food of a burgeoning population for a time. But many other countries are not so fortunate. They face instead immediate needs for population control and increased food production. An adequate supply of plant nutrients is crucial for these countries. India is one of the nations suffering the plight of low crop productivity. By tremendous effort, India, with twice the U. S. population but one-third the rice yield per acre, hopes to raise fertilizer production by 1972 to about one-fourth the present U. S. level. These are very low standards from our point of view. However, if other nations were to use potassium, nitrogen and phosphorus at the same rate as the U. S. the reserve resources would be under great pressure within a few decades.

Nutrient Supply From Soils

Looking at the intricacies of mineral uptake and use by immobile plants, one wonders how the system can work so well. Roots in soil need air and water as well as nutrients. It is remarkable that the soil can meet these needs. At first glance, soil seems only to be a loose collection of minerals in various degrees of comminution between coarse crystals and fine clays. On close inspection, these loose minerals are found held together in a fabric by the clays and residues from plant and animal life. The voids of the fabric fill with water on wetting; on standing, some of the water drains away to be replaced by air. The roots are thus in contact with both a gaseous and an aqueous phase.

Table 2. Production of maize in the United States (3)

Year	Acres Harvested millions	Production Bushels millions	Yield Bushels/ acre	Hybrids in Per cent	Nutrients used 1000 tons[a]
1933	106	2400	22.6	0.1	.....
1943	92	2970	32.2	52.4	28
1953	80	3210	39.9	86.5	294
1963	60	2100	67.6	95.0	821

[a] In Illinois.

The gas phase supplies the oxygen necessary for metabolism which drives nutrient uptake from the aqueous phase.

That nutrients are drawn from the aqueous phase, or the soil solution, is illustrated for an Iowa corn crop in Table 3. The crop, with a yield of 70 bushels/acre, takes up about 300 lbs. of potassium per acre. The solution phase to a depth of one foot in the soil contains only about 6 lbs. of potassium per acre. As the growing plants withdraw K^+ ions from the solution, they are replaced from the exchangeable K^+ of the solid phases. The K^+ content of the soil solution would be exhausted in a day at the height of the growing season if replacement did not occur. Exchangeable K^+ serves as a ballast, but it, too, is half exhausted by the end of the season. During the winter, the exchangeable K^+ is slowly built back up from the "non-exchangeable" fraction, and the solution in turn comes back to near the original concentration.

This process of cation or base-exchange affords a buffering capacity for cation nutrients in soils and is most important in plant nutrition. It was first sensed by Edmund Ruffin in his use of calcareous materials, but was only clearly expressed by J. T. Way (5) at Rothamsted in his paper published in "On the power of soils to absorb manure." The property of cation exchange is chiefly carried by acidic groups of organic residues from soil life and by the clay fractions of soils. How clays

Table 3. Potassium supply to an unfertilized Iowa corn crop (4)

Supply of K *lbs/acre*		K in solution *lbs/acre*	
Solution*	6	June 26	2
Exchange	600	July 12	1
Non-exchange	40,000	Aug. 5	0.1

* Before planting.

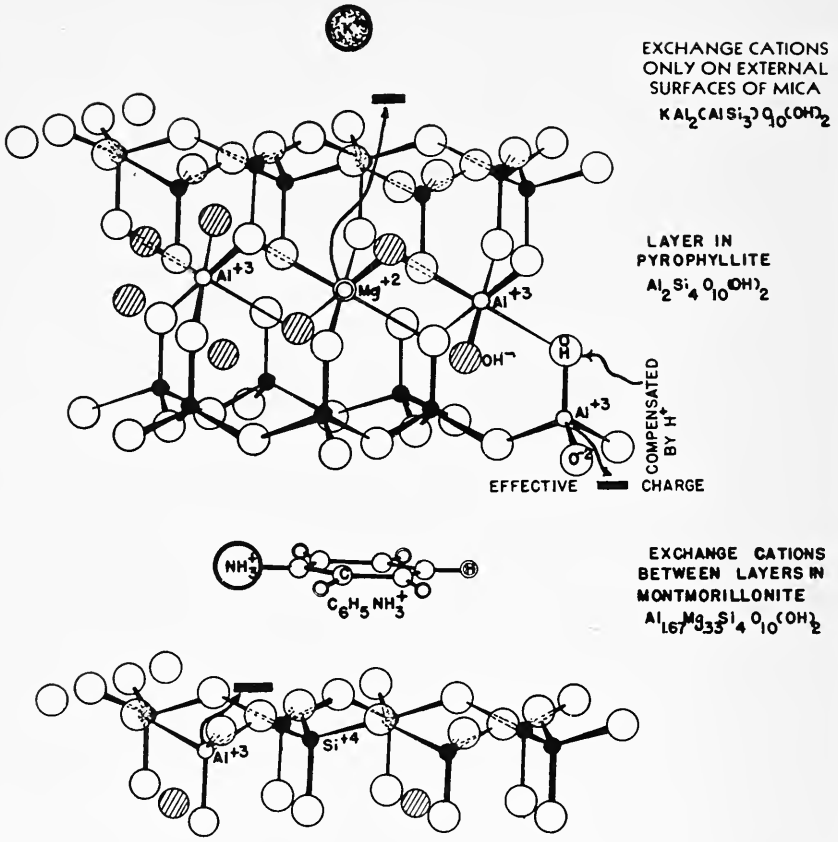


Fig. 1. Structural layers present in clay minerals related to mica and montmorillonite. Cation exchange arises from lack of balance of ionic charge within the silicate layers.

might act as acids was understood only after we had worked out their crystal structures in the decade of 1935 to 1945 (Fig. 1) (6). Then it was seen that the silicate structural sheets of the clay minerals have a negative charge because of ionic replacements in the lattice, such as Al^{+3} for Si^{+4}. Also, a crystal must terminate and the requirement for neutrality at the edge can not be met from within the lattice alone (6).

An exchange reaction can only proceed if the cation taken into the soil solution from the exchange material is replaced by another cation from the solution. Ultimately, this cation is usually a hydrogen ion derived from the plant. The clay undergoes self-reaction from attack by these hydrogen ions to yield exchangeable from non-exchangeable forms (Fig. 2). The process is not perfect, however, and where rainfall is high further neutralization by liming is necessary. The end result is a soil solution having a pH range between 4 and 8 in most soils.

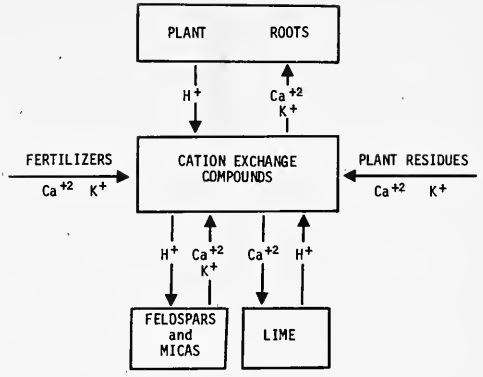


Fig. 2. Reactions of soil materials with H^+ ions from plants leading to release of nutrient cations.

Membranes and Salt Uptake

A striking feature of nutrient uptake by plants is that some salts can be accumulated against a concentration gradient. The root, moreover, resembles red blood cells and many other animal tissues in differentiating between ions as similar as K^+ and Na^+, or Ca^{++} and Mg^{++}. An example of the capacity to concentrate potassium salts is given in Table 4. Uptake of K^+ ions against concentration differences as great as 10^5 is possible. Such changes in concentration require a coupling of the uptake process to a reaction affording an adequate change in free energy. The nature of this coupling is the crux of "How Plants Take Up Nutrients."

Salt uptake by roots is essentially a membrane function. The nature of membrane transport is an active area of current biological inquiry but is largely concerned with several animal membranes, notably those of erythrocytes, frog skin, toad bladder, stomach mucosa, and kidney tubules. Mitochondrial membranes are intensively studied because of interest in the energy conversion associated with formation of adenosine triphosphate (ATP) and its coupling with ion transport into the mitochondria. I shall draw from these different areas those findings pertinent to salt uptake by plant roots.

The plant root has several tissues between the epidermis and the conducting xylem vessels leading to the shoot. In a cortical cell the

Table 4. Representative soil solution and sap concentrations

Ion	Solution *m.e./liter*	Potato Sap *m.e./liter*
K^+	0.6	70
Ca^{++}	20	13
Mg^{++}	5	42
$N(NO_3-)$	19	....

main elements in sequence are an outer cell wall with an underlying membrane (the plasmalemma), the cytoplasm, and a vacuole bounded by a membrane (the tonoplast). The rate-limiting and energy requiring steps for salt uptake are located somewhere within these structures, probably within the membranes.

The main constituents of membranes are proteins, polysaccharides, and lipids. The lipids, chiefly phospholipids, make up some 10 to 25% of the membrane and impart the essential resistance to passive permeation by ions. The proteins are in part lipoproteins and have a number of enzyme activities. Phosphatases, capable of hydrolyzing ATP and dehydrogenases, such as triosephosphate dehydrogenase have received most attention.

Electron micrographs of stained membranes show double layers in accord with the concept of protein lipid films. However, micrographs of freeze-etched fractured cell walls and of negatively stained thin sections of mitochondria suggest other possibilities including a globular type of surface arrangement. The mitochondrial membranes appear to contain tripartite units consisting of a headpiece, a connecting segment, and a base. One such assemblage has been conceived by D. E. Green and his associates (7). At all events the various observations and models to account for them indicate a complexity of membrane structure in excess of a requirement to act as a mere barrier to ion transport. Whatever the energy coupling might be for asymmetric transport, it is resident in membrane structure.

Extrapolation from our present knowledge of the kinetics of ion transport and what we know of membrane structure from electron micrographs suggests that between the globular regions of the plasmalemma and of the tonoplast there are aqueous channels. These may be held in place by interaction with membrane lipids and by interconnecting protein strands or chains. The width of these channels is of the same order as the diameter of hydrated ions, or about 0.6 nm. Enzymes are located either at the cytoplasmic interfaces or at points inside the membrane that can be reached by substrates.

Membrane structures can be studied in a functional sense by noting the conditions necessary for retention of ions. Thus, normal roots lose K^+ ions when the ambient solution has a high H^+ ion concentration ($pH<4.0$). This loss is reduced at moderate Ca^{++} ion concentrations as illustrated in Figure 3 (8). In fact, Ca^{++} ions have to be present at the root-solution interface for the functioning of the plasmalemma. About half of the Ca^{++} ions present in young soybean roots are removed in a few seconds by 0.1 N HCl without change in K content (9), indicating that Ca^{++} ions are largely external to K^+ ions. Structural membrane effects are also observed in squid axons bathed in alkaline earth salt solutions and perfused internally with alkali phosphates (10). Thus, it appears that Ca^{++} ions at the surface of the plasmalemma influence its transport properties by structural changes.

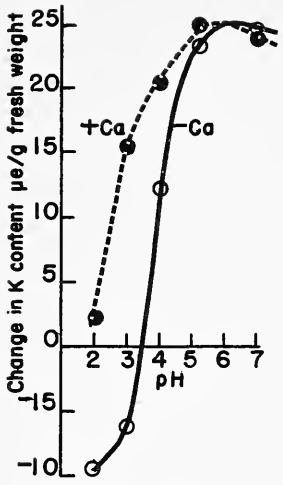


Fig. 3. Loss of K^+ ions from barley roots as a function of the pH of the ambient solution in the presence (+Ca) or absence (—Ca) of a calcium salt (18).

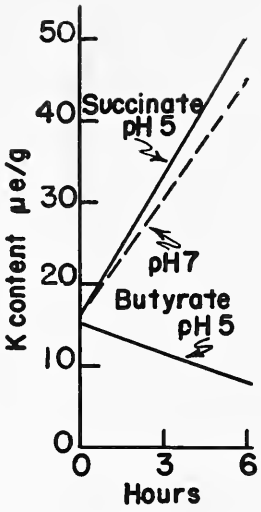


Fig. 4. Change in the K^+ ion contents of barley roots in solutions of potassium succinate or butyrate at pH 5 or 7.

An interaction effect of ions on the root membrane properties is shown with combinations of K^+, Mg^{++}, and Ca^{++} in ambient chloride solutions (11). The uptake of Mg^{++} by soybean roots, which is high from Mg^{++} salt solutions, is reduced when both Ca^{++} and K^+ ions are present (Table 5). Chloride ion uptake is enhanced in the presence of K^+ ions.

The existence of a salt-transmitting water phase in the root membrane, as well as loss of structure in the presence of acid, is shown by K^+ ion

Table 5. Effects of Ca^{+2}, Mg^{+2}, and K^+ in ambient chloride solutions for 24 hours on ionic contents of detached soybean roots at pH 5.5 (11)

Solution conc.			Ionic contents			
Ca^{+2}	Mg^{+2}	K^+	Ca^{+2}	Mg^{+2}	K^+	Cl^-
m. equiv/L			*μequiv/g fresh weight*			
0.4	....	....	6	1	50	....
....	5	....	1	43	13	0
5	5	....	8	47	43	18
5	5	1	9	11	108	55
....	....	10	2	....	32	25

retention or loss by barley roots in various organic acid salt solutions as the acidity is increased (P. C. Jackson, private communication). Thus, K^+ and butyrate are taken up from potassium butyrate solutions containing 10^{-3} eq/L KCl at pH 7, but both ions are lost if the pH is lowered to 5 (Fig. 4). Roots in equivalent succinate solutions at pH 5, on the other hand, continue to take up K^+ and succinate. The loss at pH 5 in butyrate is caused by movement of the unionized acid component into the lipoidal membrane, resulting in a structural change of the membrane which enlarges the water channels allowing K^+ and butyrate ions to leak. At pH 7, these channels are large enough for transfer of both succinate and butyrate anions. Their estimated diameter, based on the rate of transport of various anions, is about 0.6 nm. Erythrocyte membranes also contain water channels of about 0.6 nm diameter deduced by application of irreversible thermodynamic theory to their ultrafiltration properties.

Charge balance, of course, is maintained in salt transfer except for very small differences in ion transport which lead to the potential differences across membranes. This requires an equivalent movement of cations and anions, which appear to be independent exchanges of the type:

$$K^+ \underset{\text{out}}{\overset{\text{in}}{\rightleftarrows}} H^+ \quad \text{and} \quad Cl^- \underset{\text{out}}{\overset{\text{in}}{\rightleftarrows}} OH^-$$

where HCO_3^- may substitute for OH^-.

Thus, the initial rate at which excised barley roots take up K^+ does not depend on the anion supplied even though uptake rates for the anions are widely different (12).

Similarly, Cl^- is taken up at about equal rates from KCl and $CaCl_2$ solutions even though uptake rates for K^+ and Ca^{++} by barley roots differ more than 10-fold (12). Other ions necessarily involved in the charge balance are HCO_3^-, OH^-, and H^+. The changes in external H^+ (and OH^-) (Fig. 5) are of the expected sign and magnitude. When the anion is absorbed more rapidly than the cation, as with $CaCl_2$, OH^- is transferred outwards resulting in a decrease in ambient acidity which is somewhat obscured by the presence of carbonate. If both ions are transferred with about equal rates, as for $CaSO_4$, the partial processes are hidden by formation of H_2O from transferred H^+ and OH^- (Fig. 5).

Salt passage through biological membranes against osmotic gradients or ion transfer against the electrochemical potential requires coupling to an adequately energetic reaction to drive the transfer. The coupling is effected in the membrane.

Oxidation–reductions which lead to formation of ATP are adequate energy sources. Two such reactions, respiration and photosynthesis, occur respectively in the membranes of mitochondria and chloroplasts. Both the mitochondria and plastid lamellae also transfer ions. For this reason, they are ideal systems for the study of coupling reactions. The

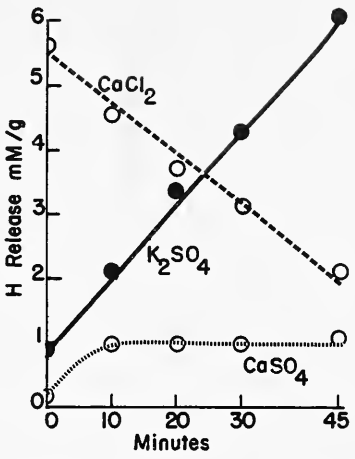


Fig. 5. H^+ ion release or absorption by barley roots in solutions of $CaCl_2$, K_2SO_4, or $CaSO_4$.

first detailed scheme was proposed by H. Lundegärdh (13, 14) who postulated a transport of anions along the respiratory chain, in the direction opposite to electron movement, from reduced substrates to oxygen. Later workers were more concerned with ways in which the electron transport could be coupled to ATP formation. Mitchell in 1961 (15, 16) suggested that oxidative phosphorylation could be coupled to H^+ appearance by the reaction:

$$ATP + H_2O + 2\ H_R{}^+ \underset{\text{synthesis}}{\overset{\text{hydrolysis}}{\rightleftharpoons}} ADP + P_i + 2\ H_L{}^+$$

where R and L refer to the two sides of a membrane and P_i is inorganic phosphate. The reaction is considered reversible. Thus, a H^+ ion gradient from L to R would drive the synthesis of ATP, while electron transfer without formation of ATP could be coupled to H^+ transfer from R to L, the overall transfer being one of: $H^+ + e^- \xrightarrow{\text{in}}$ or $e^- \underset{\text{out}}{\overset{\text{in}}{\rightleftarrows}} OH^-$.

Mitchell's scheme was supported by the observation of Jagendorf and Uribe (17) that ATP formation can be coupled to an H^+ gradient established across spinach chloroplasts. It was challenged by Chance and Mela (18) and by Tager, Veldsema-Currie, and Slater (19) who hold that ATP formation coupled to respiration is neither accompanied by the expected H^+ appearance as measured by local acidity (18) nor are the parts of the oxidation-reduction steps in the respiratory chain coupled to H^+ change (19). A detailed analysis of Mitchell's concepts has been written by R. N. Robertson (20).

From their study of the effects of respiration inhibitors and uncouplers

of ATP formation on salt transport in roots, Robertson and his coworkers (21) concluded that electron transfer in mitochondria is closely coupled to salt uptake by the plant cell. Plasmalemma and tonoplasts, however, have neither a respiratory function nor close contact with mitochondria. The coupling would have to be brought about by some change in the metabolic state of the cell accompanying a change in respiratory state of the mitochondria.

It is pertinent to inquire about the dependence of ion transport on the supply of a reactant to a membrane in erythrocytes. These cells lose Na^+ and accumulate K^+ from serum against concentration gradients but are highly permeable to Cl^-. The transport of Na^+ and K^+ is coupled, and is said to be brought about by a "pump." Some ATP is produced by glycolysis in the cells. An ATP hydrolytic enzyme (an ATPase) in the membrane is thought to drive the "pump." The ATPase requires Mg^{++}, K^+, and Na^+ for maximum activity (22). An ATPase of this type has not been observed in crop plants nor do such plants require Na^+ for growth. The concept of a "pump," however, is often used in discussing the transport of ions against an electrochemical gradient. These "pumps" are a sort of Maxwell's demon of unknown metabolism. The word "pump" is a semantic convenience equivalent to "active" transport.

Roots have surface metabolic activities. These include the appearance of H^+, OH^-, HCO_3^-, and CO_2, which were mentioned earlier. Reductive capacity can be demonstrated by placing roots in a solution of a ferric salt and a ferricyanide. Reduction of Fe^{+++} to Fe^{++} leads to precipitation of potassium ferro-ferricyanide, Turnbull's Blue. That reduction is essential for release of iron from the soil and its uptake by plants is suggested by the fact that plants with little reductive capacity are susceptible to chlorosis. A "redox-pump" mechanism for coupling energy to the transport of cations against an electrochemical gradient was advanced by Conway (23).

In the oxidation-reduction of an uncharged compound such as a quinone (Q),

$$Q + 2H^+ + 2e^- \rightleftarrows H_2Q$$

there is an associated change of the H^+ ion concentration. Conway's theory coupled the free energy change with the association of a cation with a carrier in the membrane. There is a possibility that the H^+ ion change is essential for ion transport by a membrane. If H^+ is locally changed in a membrane, possibly at its surface, two consequences would follow. First, the change would occur in a small volume in which the H^+ ion deviation from the average concentration would be very great: equivalent for example to a pH of -1. This change could be regarded as a local fluctuation. The H^+ ion would diffuse in its local gradient with a coupled diffusion of another cation. Second, the local acidity would be expected to modify the immediate surroundings, that is, aqueous

channel properties including the electrical conductivity for a particular ion would change.

The concept of local fluctuations as a means for driving ion transport can be elegantly expressed in the manner used by J. B. Schumaker and the late J. G. Kirkwood (24, 25) to account for the large dielectric polarization of protein molecules arising from fluctuations in charge and configuration of the protein molecule. An expression for an active transport current through a membrane has been derived by K. Kornacker (26). The current I_j, carried by the jth ion across a passive membrane is

$$I_j = g_j \mu_j \quad [1]$$

where g_j is the conductance and the μ_j the electrochemical potential difference across the membrane for the jth ion. If a local fluctuation of the electrochemical potential, e_j, arises in the membrane, then

$$e_j = \mu_j - \langle \mu_j \rangle \quad [2]$$

where $\langle \mu_j \rangle$ is an average value. Substitution of [2] into [1] gives

$$I_j = g_j e_j + g_j \langle \mu_j \rangle \quad [3]$$

Averaging [3] leads to

$$\langle I_j \rangle = \langle g_j e_j \rangle + \langle g_j \rangle \langle \mu_j \rangle \quad [4]$$

There is thus a component, $\langle g_j e_j \rangle$, of the average current that can be expressed after [2] as $\langle g_j \mu_j \rangle - \langle g_j \rangle \langle \mu_j \rangle$. This component is a cross correlation between the actively generated fluctuations of conductance and electrochemical potential. Local H^+ ion fluctuations would give local fluctuations of electrochemical potential for H^+ correlated with the accompanying local configuration which lead to conductance changes.

Concepts in which ATP formation and ion transport by mitochondria are dependent on changes in membrane configuration are currently being developed by D. G. Green and his associates (27 and personal communication). They observe marked changes in the tripartite units of the cristae with changes in the respiratory states of the mitochondria. The concept of a membrane acting as a cooperative unit, that is, depending for action on excitation of a region rather than a point of the membrane is drawing current attention (28). The formulation sketched in the preceding paragraphs is of this nature.

Function of Plant Nutrients

Samuel Johnson's appreciation of several major nutrient elements necessary for plant growth was illustrated in Table 1. He could not foresee with similar clearness the necessity for the minor elements: iron, manganese, boron, copper, zinc, and molybdenum, the last four being

required at levels of parts per million in plant tissue. It is easier to deal with these elements than with major nutrients when function is discussed. Each of them, with the exception of boron, is known to be required for the functioning of some enzyme (29). For example, several dehydrogenases, carbonic anhydrase, and alkaline phosphatase require zinc. Johnson was concerned about manganese, however, and this element not only has enzymatic functions, but also plays a part in oxygen evolution by chloroplasts.

Deficiency of a minor nutrient element in a crop is usually recognized by symptoms which can be confirmed by tissue analysis. Recognition and cure of deficiencies was one of the conquests of agronomists in the first half of this century.

The minor nutrient elements, with the exception of iron and manganese, are not only required in small amounts by plants but also are minor components of soils, particularly in forms available to plants. Fortunately, their levels in soils in large areas of the United States are adequate to meet crop needs. There are deficient regions, however, particularly in the sandy soils of the southern coastal plains. Copper is deficient in some of the bog soils of the midwest, and calcareous soils of the Columbia Basin are deficient in zinc. Other countries, however, are not so fortunate. Australia is notable as an old land mass with soils weathered over geologic periods. Wide areas in Australia require soil amendment with copper, zinc, or molybdenum.

Iron deficiencies in species on calcareous soils are widespread in the United States. The iron is required in heme–containing proteins such as cytochromes and non-heme–proteins, including those involved in photosynthesis, nitrogen fixation, and the respiratory linked dehydrogenases. Iron deficient soils are present in the drier western half of the United States where the difficulty is not a low iron content, but the low solubility of the soil iron compounds under alkaline conditions. Plant species show great differences in their capacity to obtain adequate iron (30, 31) from these soils. In fact, closely related varieties of soybean, maize, and sorghum differ widely in growth on such soils.

I now return to consideration of the functions of major nutrient elements. Those taken up as anions, namely sulfate, phosphate, and nitrate are incorporated as essential constituents into proteins, nucleic acids, and various other organic compounds. The proteins, cellulose, pectins, lignins, and other polymeric constituents of cell walls, membranes, and cytoplasm have carboxyl and other acidic groups so that equivalent cations must be present. Thus, balancing of charge is surely an important function of the major nutrients. Within the wall structure and the functional proteins, however, there is a further requirement for systematic arrangement of groups. The importance of Ca^{++} ions for membrane function was discussed earlier. Calcium serves this function not only in plants, but also in nerves (10), stomach mucosa, and other animal membranes, as well as for muscle action. It cannot be replaced by

Mg^{++}, but in plants can be replaced to a limited extent by Mn^{++} and possibly by hydrated Li^{+} ions. A structural function of this type depends on ionic charge and size, including the degree of hydration, which influence coordination with anions. Membrane structures lack long range order and have considerable local variability, indicating they can accommodate a moderate range of ionic compositions. Thus, a Ca^{++} salt of a protein might be insoluble and rigid while a K^{+} salt would be disordered and fluid. A combination of the two would display intermediate properties.

Repetitive, or lattice, properties of crystals discerned from diffraction patterns give a secure foundation for the structural chemistry of ionic and molecular compounds. Knowledge of structure in solution is an extrapolation from the exact information about crystals. Possible atomic arrangements in the region of closest approach to an ion are quite definite with respect to number and distance of surrounding ions or groups. Arrangements in the next region of approach (the second or outer coordination figure) are more flexible. In trying to emphasize the pervading role of structure in membrane function, I perhaps can do no better than to recall for you the determinative function of hydrogen bonding in structures of nucleic acids and proteins.

In considering the function of K^{+} ions in plants, I will paraphrase H. J. Evans and his coworkers (29, 32). Potassium is essential for all organisms. Normal plant tissue is about 0.05 M in K^{+}, deficiency symptoms appearing at about half this concentration. When enzyme activities are studied, many are found to depend on the presence of univalent cations, with maximum activity in the range of 0.01 to 0.1 M. Potassium can be replaced to a considerable extent in function by Rb^{+}, which is equal in charge and similar in hydration to K^{+}. The smaller Na^{+} ion is less effective since it is more hydrated than K^{+}.

Sorger et al. (32) examined the effect of K^{+} on the activity of pyruvic kinase which catalyzes removal of phosphate groups from phosphoenol pyruvate. An immune serum was prepared for the enzyme for examination by the micro electrophoretic precipitin technique in the presence of various electrolytes. One type of pattern, which depends in part on the tertiary structure or arrangement of parts of the protein, was obtained in the presence of K^{+}, Rb^{+}, Na^{+}, and quite a different pattern with Li^{+} and trishydroxyamino methane ($tris^{+}$). The effect of $tris^{+}$ is reversed by K^{+}.

Perhaps I have said enough about how plants get and use nutrients to give a feeling for what is and is not known. I hope also to have placed plant nutrition in its correct perspective as a practical art in food production. I realize that many of the subjects were touched on more as vignettes than in depth, but I hoped in this way to blend the whole into a pattern. Finally, I trust that if the ghost of Samuel Johnson is in the wings, he has heard something of the diversity springing from his early interests.

January 20, 1969

REFERENCES

1. Johnson, Samuel W. How Crops Grow. Orange Judd and Co. New York 1868. 391 + XVI.
2. Ruffin, Edmund. An essay on calcareous manures. Petersburg, Virginia. (1832).
3. Agriculture Statistics 1966, U.S. Government Printing Office, Washington, D. C. (1966).
4. Kelly, J. B. Composition of the displaced soil solution and exchangeable K content of high lime soils in relation to K deficiency in corn. M.S. Thesis, Iowa State University (1941).
5. Way, J. T. On the power of soils to absorb manure. J. Roy. Agric. Soc., *11:* 313 (1850).
6. Hendricks, S. B. Base exchange of crystalline silicates. Ind. and Eng. Chem. *37:* 625-639 (1945).
7. Pennington, J. T., R. A. Harris, J. Asai, and D. E. Green. The conformational basis for energy transformation in membrane systems. I. Conformational changes in mitochondria. Proc. Nat. Acad. Sci. *59:* 624-631 (1968).
8. Jacobson, L., D. P. Moore, and R. J. Hannapel. Role of calcium in absorption of monovalent ions. Plant Physiol. *35:* 352-358 (1960).
9. Leggett, J. E. and W. A. Gilbert. Localization of the Ca-mediated apparent ion selectivity in the cross sectional volume of soybean roots. Plant Physiol. *42:* 1658-1664 (1967).
10. Watanabe, A., I. Tasaki, and L. Lerman. Bi-ionic action potentials in squid giant axons internally perfused with sodium salts. Proc. Nat. Acad. Sci. *58:* 2246-2252 (1967).
11. Leggett, J. E. and W. A. Gilbert. Magnesium uptake by soybeans. Plant Physiol. (In press.)
12. Jackson, P. C. and H. R. Adams. Cation-anion balance during potassium and sodium absorption by barley roots. J. Gen. Physiol. *46:* 369-386 (1963).
13. Lundegärdh, H. Absorption, transport, and exudation of inorganic ions by roots. Ark. Bot. 32A: 1-139 (1945).
14. Lundegärdh, H. Spectroscopic evidence of the participation of the cytochrome-cytochrome oxidase system in the active transport of salts. Arkiv. Kemi. *3:* 69-79 (1951).
15. Mitchell, P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature *191:* 144-148 (1961).
16. Mitchell, P. Chemi-osmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. *41:* 445-602 (1966).
17. Jagendorf, A. T. and E. Uribe. ATP formation caused by acid base transition of spinach chloroplasts. Proc. Nat. Acad. Sci. *55:* 170-177 (1966).
18. Chance, B. and L. Mela. Proton movements in mitochondrial membranes. Nature *212:* 372-376 (1966).
19. Tager, J. M., R. D. Veldsema-Currie, and E. G. Slater. Chemie-osmotic-theory of oxidative phosphorylation. Nature *212:* 376-379 (1966).
20. Robertson, R. N. Protons, electrons, phosphorylation, and active transport. Cambridge Univ. Press. London-New York (1968).
21. Atkinson, M. R., G. Eckermann, M. Grant, and R. N. Robertson. Salt accumulation and adenosine triphosphate in carrot xylem tissue. Proc. Nat. Acad. Sci. *55:* 560-564 (1966).
22. Skou, J. C. Enzymatic aspects of active linked transport or Na^+ and K^+ through the cell membrane. Progr. in Biophys. *14:* 131-168 (1964).
23. Conway, E. J. Evidence for a redox pump in the active transport of cations. Int. Rev. Cytol. *4:* 347-355 (1955).
24. Kirkwood, J. G. and J. B. Schumaker. The influence of dipole moment fluctuations on the dielectric increment of proteins in solutions. Proc. Nat. Acad. Sci. *38:* 855-862 (1952).

25. Kirkwood, J. G. and J. B. Schumaker. Forces between protein molecules in solution arising from proton charge and configuration. Proc. Nat. Acad. Sci. *38:* 863-871 (1952).
26. Kornacker, K. Theory of inherently macroscopic processes, with application to heat and active transport. Nature *219:* 1283-1284 (1968).
27. Harris, R. A. J. T. Penniston, J. Asai, and D. E. Green. The conformational basis of energy conservation in membrane systems. II. Correlation conformational change and functional states. Proc. Nat. Acad. Sci. *59:* 830-835 (1968).
28. Changeux, J. P., J. Thiery, Y. Tung, and C. Kittel. On the cooperativity of biological membranes. Proc. Nat. Acad. Sci. *57:* 335-341 (1967).
29. Evans, H. J. and G. J. Sorger. Role of mineral elements with emphasis on the univalent cations. Ann. Rev. Plant Physiol. *17:* 47-76 (1966).
30. Brown, J. C. Genetic variants as factors affecting the nutrition and physiology of plants. "Isotopes in Plant Nutrition and Physiology." Int. Atomic Energy Agency, Vienna. 413-420 (1967).
31. Brown, J. C., C. R. Weber, and B. E. Caldwell. Efficient and inefficient use of iron by two soybean genotypes and their isolines. Agron. J. *59:* 459-462 (1967).
32. Sorger, G. J., R. E. Ford, and H. J. Evans. Effects of univalent cations on the immuno-electrophoretic behavior of pyruvic kinase. Proc. Nat. Acad. Sci. *54:* 1614-1621 (1965).

How Legumes Fix Nitrogen*

Harold J. Evans

Nitrogen is the most abundant element in our atmosphere and is a major constituent of all living organisms. Higher plants contain an average of three per cent, microbes eight per cent and animals ten per cent nitrogen in their dry tissues (12). Compounds of nitrogen useful for the nutrition of living things, are made available by several different processes varying greatly in magnitude and importance. During thunderstorms small amounts of atmospheric nitrogen gas are converted to nitric acid by electrical discharges (46) and some ammonium nitrogen is reported (46) to be derived from a few types of rocks during the weathering process. An increasing quantity of the nitrogen from the air is industrially reduced to ammonia each year by the Haber-Bosch process. The precise quantities of nitrogen supplied to the biosphere by all the various processes is not accurately known, but it is generally agreed (2) that the biological fixation of nitrogen gas is of major importance, accounting for some hundred million tons of nitrogen fixed per year (20). Less than ten per cent of the total is estimated to be contributed by processes exclusive of biological nitrogen fixation.

Relatively few organisms possess the capacity to convert atmospheric nitrogen into nitrogenous compounds. Among these are free-living bacteria such as *Azotobacter, Beijerinckia, Chromatium, Clostridium,* and *Klebsiella* (16). Certain species of blue-green algae fix nitrogen and examples of these are *Anabaena, Calothrix, Nostoc* and *Tolypothrix* (16). During recent years Bond (11) has pointed out that several non-leguminous angiosperms, including species of *Alnus, Myrica, Hippophae, Casuarina* and *Ceanothus,* possess root bearing nodules with an endophyte that are capable of symbiotic nitrogen fixation. For centuries agriculturists have recognized the ability of leguminous plants to improve the productivity of the soils, and now we know that this property is associated with the remarkable capacity of the legume-Rhizobium association to convert atmospheric nitrogen into ammonia. Some thirteen

* This paper was prepared during a sabbatical leave at the A.R.C. Unit of Nitrogen Fixation at The University of Sussex. The author is grateful to the Rockefeller Foundation, to Oregon State University and to The National Science Foundation for support during this period.

thousand species of *Leguminosae* have been described, and it is stated that most of these are nodulated (2, 16). In the United States alone, leguminous crops have been estimated (16) to fix 5.5 million tons of nitrogen per year. It is clear therefore that leguminous plants, growing in symbiosis with appropriate *Rhizobium* species, are of great economic importance in the conversion of atmospheric nitrogen to a form that can be used efficiently for the nutrition of living things.

Samuel W. Johnson (28), founder of The Connecticut Agricultural Experiment Station, certainly appreciated the importance of symbiotic nitrogen fixation by legumes, because his book entitled, *How Crops Feed* which was published in 1870, presented an accurate summary of the available information on the fixation of nitrogen by leguminous plants. This included a description of the early experiments of Boussingault, reported in 1837, from which he concluded initially that nitrogen gas was not fixed by leguminous species. In 1838, however, Boussingault (13) published additional results indicating that legumes but not cereals accumulated more nitrogen during their development than was supplied to them in the nutrient medium. He did not appreciate the biological differences between legumes and non-legumes and thus was not able to accurately interpret his findings. In Johnson's book there is also an account of the researches of Ville, who claimed that both legumes and non-legumes could utilize nitrogen from the atmosphere. Johnson (28) presented a clear description of the complex experiments of Lawes, Gilbert and Pugh, in which both legumes and non-leguminous plants were grown in calcined soil under conditions where contamination by fixed forms of nitrogen was highly improbable. From the data collected they were forced to conclude that neither legumes nor non-legumes possess a capacity to utilize nitrogen gas, but they failed to recognize that micro-organisms normally present in soils were eliminated by heating the soil and thus had no opportunity to participate in symbiotic nitrogen fixation.

Professor Perry Wilson (47) has presented an informative account of the establishment of the early experiment stations in the United States, including interesting facts about the first one at Wesleyan University in Connecticut under the directorship of W. O. Atwater. Atwater conducted experiments on the nutrition of peas and published results in 1885 (4) showing fairly convincingly that tissues of pea plants contained more nitrogen at harvest than was supplied to them in the nutrient medium. He believed that this increase must have arisen from nitrogen of the atmosphere. Atwater was aware that his results were not in agreement with those of certain authorities in the field and was tempted to seek non-biological explanations for his observations.

The capability of leguminous plants to fix atmospheric nitrogen was not fully appreciated until the results of the classical experiments of Hellriegel and Wilfarth (25) were published in 1888. They made the important assumption that nodules on the roots of leguminous plants were induced by bacteria with a capability of fixing atmospheric nitro-

gen and that leguminous plants lacking nodules behave like non-legumes utilizing fixed nitrogen compounds but not nitrogen from the air. They grew both legumes and non-legumes in a non-sterile medium and in another sterile medium, to which was added an extract of garden soil. Their results fully substantiated the original assumptions and provided definitive evidence that nodulated legumes fix atmospheric nitrogen and that non-nodulated legumes and non-legumes lack this capability. This dramatic discovery was followed by experiments of Schloesing and Laurent (42) who showed that the amount of nitrogen incorporated into the tissues of nodulated peas was approximately equal to the quantity of gaseous nitrogen lost from the atmosphere around them. The matter was further clarified when Beijerinck in 1888 (5) isolated pure cultures of bacteria that caused the formation of nodules on the roots of certain leguminous plants.

Development of Nodules

Now we know that different species of *Rhizobium* invade roots of certain groups of legumes and are responsible for the initiation of root nodules. *R. meliloti* causes the formation of nodules on plants such as sweet clover (*Melilotus*) and alfalfa (*Medicago*); *R. trifolii* is responsible for nodulation of certain clovers, for example, red clover (*Trifolium pratense*) and white clover (*T. repens*); *R. leguminosarum* nodulates peas (*Pisum sativum*) and broad beans (*Vicia faba*); *R. phaseoli* causes nodules on roots of garden beans (*Phaseolus vulgaris*); *R. japonicum* is relatively specific for the nodulation of soybeans (*Glycine max*), and *R. lupini* effectively nodulates various species and strains of lupines (*Lupinus*). Within each *Rhizobium* species, strains exist that exhibit varying degrees of effectiveness in nodulation and nitrogen fixation when combined with appropriate species (or strains) of leguminous plants within a particular cross-inoculation group.

The infection of legume roots by *Rhizobium* has been reviewed in detail by Nutman (39) and initial aspects of the process are illustrated in Figure 1 (45). *Rhizobium* cells are reported to collect near the tip of a root hair of the host plant as a result of the secretion of nutritive substances. Secretion of a polysaccharide slime by the bacteria induces the plant to secrete polygalacturonase which aids in the entry of the bacteria. The specificity of particular *Rhizobium* species for certain leguminous plants is believed to be related to the type of polysaccharide produced by the bacteria. The production of growth substances by the bacteria causes the root hair to curl and an infection thread of plant origin is formed. The bacteria within the infection thread proliferate as it passes through the cortical cells of the root. When the infection thread reaches one of the few tetraploid cells in the inner cortical region, the bacteria are released and in some unknown way both tetraploid cells and adjacent diploid cells are induced to meristematic activity.

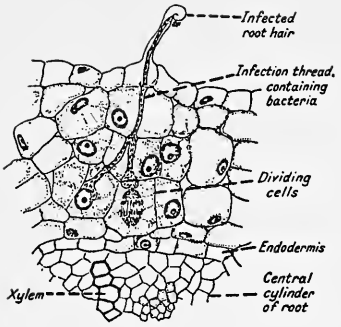


Fig. 1. An illustration of the infection of an alfalfa root (*Medicago sativa*) by *Rhizobium meliloti*. From Thornton (45).

As these cells divide and the nodule increases in size the bacteria divide and eventually are enclosed in groups of two, three or more within a membrane envelope (6). In many *Rhizobium* species the bacteria within the nodules assume odd shapes and are referred to as bacteroids rather than bacteria.

Within the membrane envelopes surrounding bacteroids is a relatively high concentration of a red pigment originally identified by Kubo (37) as a hemoglobin. The hemoglobin content of nodules is positively correlated with the capacity to fix nitrogen gas (46). Kubo (37) originally suggested that nodule hemoglobin was involved in the transport of oxygen to the bacteroid cells. This interpretation has been questioned (43) however, because the concentration of carbon monoxide that blocks oxygen transport by hemoglobin fails to interfere with the stimulation of respiration by this pigment. Suggestions have been made (1) that hemoglobin forms a complex with nitrogen gas. Bergersen and Wilson (10) have demonstrated that the spectrum of ferrohemoglobin is altered upon the addition of nitrogen gas, and infer that the pigment in some way is related to nitrogen reduction. Although many researchers agree that the vigor of nitrogen fixation is consistently correlated with the content of hemoglobin in nodules the precise role of the pigment remains obscure.

The Site of Nitrogen Fixation

The classical work of Wilson (48, 49) revealed that hydrogen was a specific inhibitor of nitrogen fixation by red clover plants, and Hoch et al. (26, 27) demonstrated that soybean nodules evolved hydrogen during nitrogen fixation. This and other evidence provided a basis for linking the process of nitrogen fixation with hydrogen metabolism. The relationship between hydrogen and nitrogen metabolism could not be understood however until cell-free extracts of nitrogen fixing organisms could be prepared.

By use of the sensitive ^{15}N technique, Aprison et al. (3) established that excised soybean nodules fix nitrogen for a period of about two hours.

The rate of fixation in sliced nodules was greatly reduced and fixation ceased when nodules were crushed (15, 23). On the basis of kinetic experiments in which soybean nodules were exposed to $^{15}N_2$ then fractionated into bacteroids, membranes, and soluble proteins, Bergersen (6) concluded that the site of nitrogen fixation was the membranes that surround bacteroids. These observations together with those (10) revealing a spectral change in nodule hemoglobin that was dependent upon nitrogen gas, provided the basis for a hypothetical mechanism of nitrogen fixation in nodules. Bergersen (6) proposed that cytochromes in the plasma membranes of bacteroids were reduced by metabolic processes and that electrons were transferred from them to the hemoglobin pigment surrounding the bacteroids. Ferrohemoglobin was believed to pass electrons to the nitrogen fixing sites in the membranes surrounding bacteroids. Klucas and Burris (30) recently have conducted kinetic experiments similar to those described by Bergersen and shown that the ^{15}N concentration was greatest in the soluble fraction of nodules and not in the membranes. Kennedy (29) exposed serradella (*Ornithopus sativus*) nodules to $^{15}N_2$ and then determined the extent of incorporation of the isotope in several nodule fractions and concluded that bacteroids (maintained in a sucrose medium) contained the highest concentration of the isotope. From these results Bergersen's hypothesis is no longer tenable and now has been discarded by him (8).

Bergersen (7) has utilized a special press under anaerobic conditions for the preparation of a nodule brei that retains nitrogen fixing capability. The activity of this system was not dependent on an exogenous reductant nor an ATP supply. Additional research by Bergersen and Turner (8) established that the nitrogen fixing activity in the brei was associated with the bacteroids and that nodule hemoglobin was not essential. Koch, Evans, and Russell (35) independently arrived at the same conclusion by use of different methods.

After the development of a new sensitive method of assaying nitrogenase in 1966 our laboratory (34) embarked upon a systematic research program to obtain nitrogen fixing cell-free extracts of legume nodules. Schöllhorn and Burris (40, 41) and Dilworth (19) had made the important basic discovery that nitrogenase preparations from *Clostridium pasteurianum* not only catalyzed the reduction of nitrogen but also the reduction of acetylene. Dilworth (19) identified ethylene as the reaction product and proved beyond doubt that the nitrogenase system was responsible for the reduction of both nitrogen gas and acetylene. After the publication of these results Hardy and Knight (23) used the hydrogen flame method for detection of products of the nitrogenase reaction that had been separated by gas chromatography, and suggested the use of the acetylene reduction method for measurement of nitrogenase. By use of the acetylene assay Koch and Evans (34) measured ethylene production from acetylene by soybean nodules in a reaction vial containing 0.65 atm. of argon, 0.25 atm. of oxygen and 0.1 atm. of

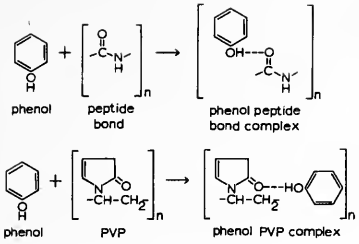


Fig. 2. Proposed reactions of phenols with a peptide bond and with a unit of polyvinylpolypyrrolidone.

acetylene. The conditions found to be necessary for the reaction indicated that nitrogenase was involved in the reduction. Stewart et al. (44) used the acetylene method to investigate the distribution of nitrogen fixing algae in lakes and more recently Hardy et al. (22) have carried out a comprehensive study of the relationship of acetylene reduction to nitrogen fixation under both laboratory and field conditions. Already it is clear that the basic discoveries of Dilworth (19) and Schöllhorn and Burris (40, 41) have enormous application to biological nitrogen fixation problems.

Another important technique that proved to be crucial in the preparation of active nodule breis was the use of insoluble polyvinylpolypyrrolidone (PVP). Loomis and Battaile (38) presented a detailed account of the usefulness of this material in the preparation of enzyme extracts from plant tissues known to contain large quantities of phenolic compounds. As illustrated in Figure 2, phenolic materials in macerated plant tissues may react with peptide bonds of proteins and denature them. If tissues are macerated under anaerobic conditions in the presence of a reductant and a high concentration of PVP, phenols form complexes with PVP through hydrogen bond reactions. The insoluble PVP-phenol-complex then is removed by filtration, leaving an active extract. Since chromatographic procedures in our laboratory revealed high contents of phenolics in legume nodules a decision was made to attempt to prepare active breis and cell-free nitrogenase by macerating nodules under anaerobic conditions in a medium that included PVP and ascorbate. The merits of this procedure are obvious from the acetylene reducing activities of breis prepared by different methods (Table 1). When both PVP and ascorbate were omitted in the preparation medium practically no acetylene was reduced by the brei. In contrast when both of these compounds were used in the procedure the brei from 2.5 gm of nodules produced 14 μmoles of ethylene in one hour. Furthermore a bacteroid fraction could be obtained that retained practically all of its acetylene reducing activity, provided that either the soluble fraction or certain substrates such as succinate or β-hydroxybutyrate were added (Table 2). Nodule hemoglobin had no activity alone and it was not necessary to add it to the bacteroids in order to obtain acetylene reduction. This evidence and that of Bergersen and Turner (8) clearly proved that nitrogenase is located in nodule bacteroids.

Table 1. The effect of polyvinylpolypyrrolidone (PVP) and ascorbate in the preparation medium on the acetylene reducing capacity of a soybean nodule brei

Each reaction mixture contained 10 ml of nodule brei prepared from 2.5 gm of soybean nodules. The gas volume (22 ml) above the mixture was composed of 0.25 atm of O_2, 0.65 atm of argon and 0.1 atm of acetylene. From data of Koch, Evans and Russell (35).

Preparation of brei	Ethylene produced (μmoles/hr)
With PVP	7.50
Without PVP	0.08
With PVP and 0.3 M ascorbate	14.11
Without PVP, with 0.3 M ascorbate	0.48

Preparation of Cell-free Extracts

In the first experiment (18) in which cell-free nitrogen fixation was demonstrated with extracts of *Clostridium pasteurianum*, pyruvate was utilized as a source of metabolic energy. The breakdown of this substrate proved to generate reducing power through the intermediate ferredoxin. Pyruvate also supplied acetylphosphate which served as a source of energy for maintenance of the ATP supply. Pyruvate failed to support nitrogen fixation by extracts of *Azotobacter*, but Bulen et al. (14) discovered that cell-free extracts of this aerobe fixed nitrogen at

Table 2. Acetylene reducing activity of fractions derived from a brei of soybean nodules

Preparation of the brei and the assay for ethylene has been described by Koch, Evans and Russell (35). The brei (from 3.9 g of nodules) in each reaction mixture was separated where indicated into bacteroids and soluble proteins by centrifugation at 30,000 $\times$ g for 20 minutes. Bacteroids were washed in 50 ml portions of a buffer solution containing 0.2 M ascorbate and 0.02 M phosphate buffer at pH 7.2. The succinate concentration in the final reaction mixture was 0.01 M. Other details of the experiment are essentially the same as described in Table 1. Unpublished data of Burton, Koch and H. J. Evans, 1967.

Treatment	Ethylene (μmoles/hr)
Brei control	25.3
Brei centrifuged, bacteroids washed, recombined with supernatant	26.5
Bacteroids washed, suspended in ascorbate-phosphate buffer	13.5
Bacteroids washed, succinate added	21.8
Soluble proteins from brei	0.01
Brei boiled	0.01

a rapid and reproducible rate when sodium hydrosulfite and an ATP generating system were included in anaerobic assay mixtures. When this information became available several researchers (16, 17) added either pyruvate, or sodium hydrosulfite and an ATP generating system to nodule breis or extracts but no nitrogen fixing preparations that were dependent upon these additions were observed.

The essential methodology (35, 36) that proved to be necessary for the preparation of nodule bacteroids that could reduce acetylene or nitrogen gas also proved to be crucial in the preparation of active cell-free extracts of bacteroids. In brief the following steps were involved in the process. Soybean nodules (100 to 200 gm samples from six-week-old plants) were placed in a Waring blender vessel containing phosphate buffer ascorbate and PVP. The mixture was gassed thoroughly with nitrogen or argon and the nodules were macerated in the jar while a stream of nitrogen flowed through it. Solid debris and PVP were anaerobically removed from the macerate by squeezing the liquid through bolting cloth inside a nitrogen filled glove box. In all subsequent operations anaerobic conditions were maintained by working in a glove box or by gassing reagents and vessels with nitrogen. Bacteroids in the nodule brei were collected by centrifugation, broken by use of a French press, then centrifuged at 40,000 g for one hour to obtain a dark brown cell-free extract.

Since the crude extract contained endogenous ammonia and other compounds that interfered with the direct measurement of ammonia synthesis, Bio-gel chromatography under anaerobic conditions, was employed as an initial purification step. Crude extracts or chromatographed preparations remained active for a few hours at 4 to 9°C, but retained most of their activities for several months when they were frozen and stored in liquid nitrogen.

Properties of Bacteroid Nitrogenase

Essential components. Use of the acetylene assay quickly revealed that crude extracts of soybean nodule bacteroids reduced acetylene to ethylene. The reaction required anaerobic conditions, sodium hydrosulfite and an ATP generating system (35, 36). Both the crude and chromatographed extracts also actively reduced nitrogen gas to NH_3 (Table 3). In fact, in the first experiment in which an extract capable of acetylene reduction was tested, sufficient NH_3 was produced for direct measurement either by titration or Nesslerization after microdiffusion into acid. Conclusive results also were obtained by exposure of reaction mixtures to $^{15}N_2$ and determination of the incorporation of the isotope into NH_3 (Table 3). The system was dependent upon N_2, the ATP generating system and sodium hydrosulfite. The reaction rate was increased by the addition of $MgCl_2$ and creatine phosphokinase (both components of the ATP generating system) but complete de-

Table 3. The components necessary for cell-free nitrogen fixation
The duration of incubation in each of these experiments was an hour. For details of the experimental procedure see Koch, Evans and Russell (36).

Reaction	Expt. I NH_3 formed (mμmoles/ min/mg protein)	Expt. II $^{15}N_2$ fixed (atom % excess)	Expt. III $^{15}N_2$ fixed (atom % excess)
Complete	3.5	2.283	3.891
Without N_2 or $^{15}N_2$	0.2	0.002	0.005
" ATP system	0.0	0.004	0.002
" $Na_2S_2O_4$	0.2	0.003	0.002
" ATP	0.0	.....	.....
" $MgCl_2$	1.4	.....	.....
" creatine phosphate	0.4	.....	.....
" creatine phosphokinase	0.6	.....	.....

pendence on these additions was not demonstrated with preparations from the early stages of purification. Application of the procedure for preparation of cell-free extracts of soybean nodule bacteroids to nodules from lupines and serradella also yielded positive results (Table 4), provided that sodium hydrosulfite and the ATP generating system was present and oxygen damage was prevented.

Bergersen and Turner (8) have investigated the properties of nitrogenase in extracts from soybean nodule bacteroids prepared by a method similar to that of Koch, Evans and Russell (36) except that soluble PVP was used instead of the insoluble preparation. Nitrogenase activity remained in the supernatant liquid after centrifugation at 100,000 × g for 30 minutes and was dependent upon sodium hydro-

Table 4. Nitrogenase activities of cell-free extracts of bacteria from nodules of serradella (Ornithopus satinus) and lupine (Lupinus luteus)
The experimental procedure was essentially the same as that described for experiment I, Table 3 (Koch, Evans and Russell [36]) with the exception that crude extracts were utilized. The results have been corrected for the NH_4^+ content of the extracts. Unpublished experiments of S. Russell and H. J. Evans, 1967.

Reaction	N_2 reduced (mμmoles/mg protein/min) Serradella	Lupine
Complete	3.55	5.03
—ATP system	0.02	0.03
—$Na_2S_2O_4$	0.05	0.50

sulfite and on an ATP generating system. Bergersen and Turner (9) reported evidence for cold lability of the enzyme but this property of bacteroid extracts has not been observed in our laboratory.

Effects of different gases. Bacteroid nitrogenase is rapidly inactivated by exposure to oxygen during preparation. The enzyme system also is inactivated if exposed to oxygen during the assay procedure. An excess of sodium hydrosulfite in assay mixtures prevents enzyme loss by combining with traces of oxygen that remain in mixtures after gassing with nitrogen.

Assay of the bacteroid nitrogenase under a series of different partial pressures of nitrogen revealed maximal activity at about 0.25 atm and one-half maximum activity at a pN_2 of 0.053 atm (36). According to Burris (15) one-half maximum rate of nitrogen fixation by sliced soybean nodules was observed at a pN_2 of 0.025 atmosphere.

In agreement with the classical work of Wilson et al. (48, 49) showing that hydrogen gas was a specific inhibitor of nitrogen fixation, experiments in which cell-free extracts were exposed to hydrogen revealed marked inhibition. At a pN_2 of 0.25 atm, the addition of 0.25 atm of hydrogen inhibited 67%. The inhibition appeared to be of the competitive type. When hydrogen was added at 0.05 atm, a Ki for hydrogen of 0.16 atm was calculated. Carbon monoxide also is reported (9) to be a powerful competitive inhibitor of nodule nitrogenase.

Gas Evolution

In 1960 Hoch et al. (27) demonstrated that soybean nodules evolve hydrogen gas and catalyze an exchange between H_2 and D_2 and endogenous hydrogen donors. The release of H_2 by nodules was inhibited by N_2 and this was believed to be related in some way to the nitrogen fixing reaction. These results together with those of Bulen et al. (14) provided the basis for an experiment designed to measure gas evolution during nitrogen fixation by cell-free nodule extracts. Reactions containing active extract, sodium hydrosulfite, an ATP generating system, and buffer were placed in Warburg flasks and allowed to react under an atmosphere of nitrogen. Both nitrogen fixation and the change in volume of gas above the reaction were determined (Fig. 3). Under nitrogen 3 μmoles of NH_3 (amounting to 9 μEq of reductant) were formed and 24 μl of gas were evolved. This was identified as hydrogen and is equivalent to 2.2 μEq of reductant. The reaction carried out under argon failed to fix nitrogen but evolved 152 μl of hydrogen, a volume that would require about 13.6 μEq of reductant for formation. Since a total of 11.2 μEq of reductant were utilized under conditions where nitrogen was reduced it would appear that reductant was consumed at about the same rate under either nitrogen or argon. In control experiments lacking the ATP generating system, no gas was evolved. These results are in

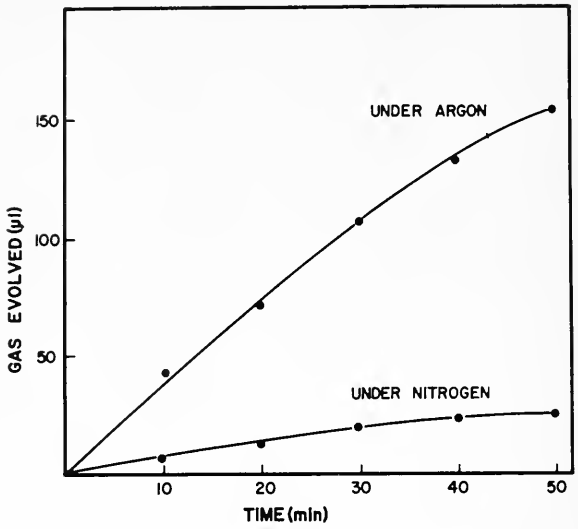


Fig. 3. Nitrogenase-dependent evolution of gas under either N_2 or argon (after Koch, Evans and Russell [36]. The complete reaction mixture (3 ml) in a Warburg vessel contained chromatographed bacteroid extract (12 mg of protein), sodium hydrosulfite, buffer, and the ATP generating system. Prior to the addition of the extract, flasks were flushed thoroughly with N_2 or argon as indicated. After 50 minutes NH_3 synthesis was measured, after microdiffusion, by Nesslerization.

general agreement with those of Bulen et al. (14) that were obtained by use of extracts of *Azotobacter vinelandii.*

Fractionation

A review (21) of the properties of nitrogenase from *Azotobacter vinelandii* and *Clostridium pasteurianum* reveals that the enzyme system may be resolved into two components both of which are required for activity. One fraction with a molecular weight of 100,000 to 120,000 contains molybdenum, acid labile sulphur, and non-heme iron. The other fraction has a molecular weight in the range of 40,000 to 60,000 and also contains acid labile sulphur and non-heme iron but shows no increased concentration of molybdenum.

Since nutritional experiments conducted during the past 30 years have consistently indicated a specific role of molybdenum in nitrogen fixation by legumes and other nitrogen fixing organisms (17), it was of great interest to fractionate soybean bacteroid nitrogenase and establish properties of the fractions including metal contents. Initial ex-

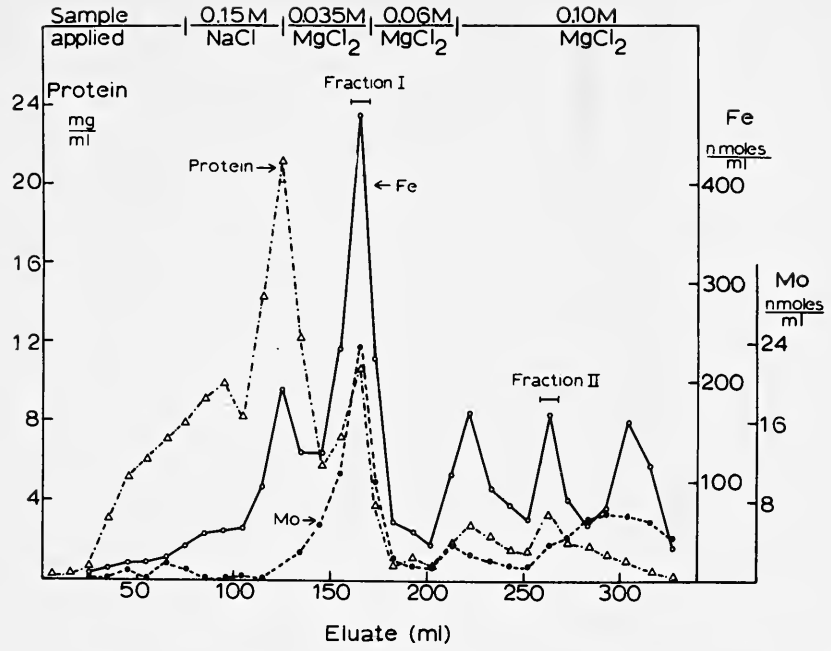


Fig. 4. Chromatographic separation of bacteroid nitrogenase in two components (after Klucas et al. [33]). Crude bacteroid extract (950 mg of protein) was first fractionated with protamine sulfate then chromatographed anaerobically on a DEAE column using solutions of NaCl and $MgCl_2$ to elute the proteins.

periments (33) involving a heat treatment, protamine sulfate precipitation then anaerobic chromatography on DEAE cellulose resulted in appreciable purification but failed to resolve two distinct components. When the experiment was repeated varying some of the initial purification steps and using an anaerobic DEAE column equilibrated with Tris buffer, two distinct fractions essential for enzyme activity were removed by elution with solutions of NaCl and $MgCl_2$ (Fig. 4). Fraction I contained appreciable non-heme iron and molybdenum but only non-heme iron was concentrated in fraction II. The specific activities (Table 5) of fractions I and II measured individually were 0.0 and 6.0 respectively but a combination of equal volumes of these fractions had specific activity of 73 μmoles of N_2 reduced per minute per mg of protein in fraction II. Activity was expressed on the basis of fraction II because fraction I was added in excess (Table 5). Additional experiments accomplished a complete resolution of the nitrogen reducing activities of the two fractions and demonstrated the expected activity response when either of the fractions was titrated against the other (Fig. 5). The ratios of the rates of acetylene reduction to the rates of nitrogen fixation by

Table 5. A summary of some properties of fractions of soybean (Glycine max) nodule nitrogenase (after Klucas et al. [33])

Enzyme preparation	Nitrogenase (mμmoles N_2 fixed/ mg protein/ min)	Fe (mμmoles/ mg protein)	Mo (mμmoles/ mg protein)
Crude extract	9	7.9	1.1
Fraction 1	0	44	2.2
Fraction 2	6	50	1.0
Fraction 1 + 2	73*	....	....

* Equal volumes of fractions 1 and 2 were used but activity was expressed on the basis of the protein in fraction 2 since it was limiting in the reaction.

fractions varying greatly in extent of purity were approximately constant at a value near 3.0. Although further purification is necessary it seems clear that nitrogenase from legume nodules is similar in most respects to the nitrogenase that has been purified from other sources.

Natural Reducing Systems

Oxidizable substrates available to nitrogen-fixing organisms must provide both the reducing power and the ATP that is essential for nitrogen fixation. Pyruvate serves both of these purposes in reactions containing crude extracts of *Clostridium pasteurianum* but the only reductant that initially was proven to be effective in assay systems containing extracts of *Azotobacter* or nodule bacteroids was sodium hydrosulfite. When $Na_2S_2O_4$ is employed as a reductant, an ATP generating system also is necessary to replace the natural source of ATP that would be maintained *in vivo* through reactions associated with oxidative phosphorylation or glycolysis. Our laboratory has devoted considerable effort toward the elucidation of natural electron transport processes that might support nitrogen fixation or nitrogenase-dependent acetylene reduction.

Rhizobium species are known (24) to accumulate poly-β-hydroxybutyrate. Mr. Peter Wong in our laboratory has isolated and identified pure poly-β-hydroxybutyrate from soybean nodule bacteroids and shown that 30 to 40% of the dry weight of bacteroids may be accounted for as this storage product. Furthermore, extracts of soybean nodule bacteroids contain an unusually active β-hydroxybutyrate dehydrogenase that currently is being investigated by Mr. Peter Wong. This information together with the observation (31) that extracts of nodule bacteroids and other nitrogen fixing organisms actively catalyze the reduction of certain dyes such as benzyl viologen (redox potential of −0.32 volt) by NADH provided a basis of hypothetical electron transport sequence from substrate to nitrogenase. As illustrated (Table 6) a complete anaerobic

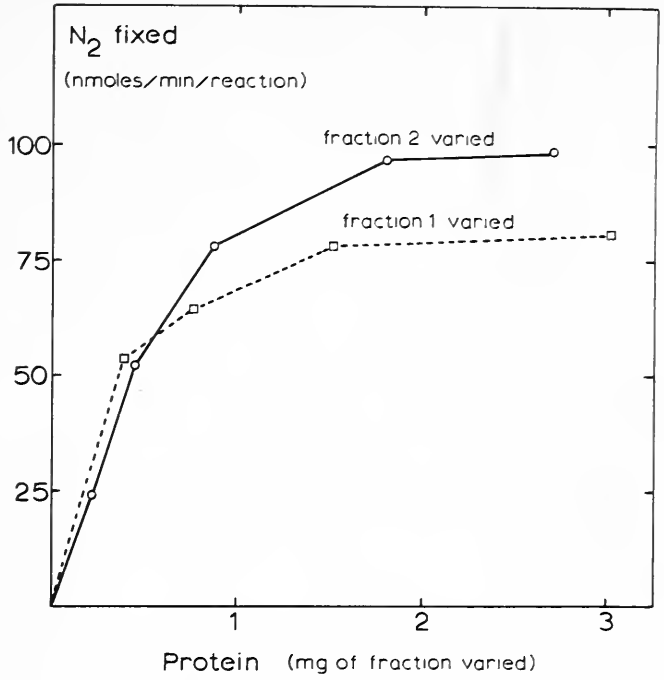


Fig. 5. Nitrogenase activity as influenced by different combinations of fractions 1 and 2 (after Klucas et al. [33]). The fractions were prepared from an extract of nodule bacteroids by a procedure involving polypropylene glycol precipitation and then chromatography on an anaerobic DEAE column using a procedure similar to that described in Figure 4. When fraction 2 was varied all reactions contained a constant amount (1.5 mg protein) of fraction 1. When fraction 1 was varied all reactions contained a constant amount of fraction 2 (0.9 mg protein).

reaction mixture containing active bacteroid nitrogenase, an ATP generating system, benzyl viologen, a catalytic amount of NADH or NAD and β-hydroxybutyrate catalyzed the reduction of acetylene to ethylene at an appreciable rate. Vigorous activity was dependent upon all these additions. All the evidence was consistent with the conclusion that nitrogenase was responsible for the catalysis. Furthermore (Fig. 6) the complete reaction proceeded at a linear rate for over 30 minutes after an initial lag caused by traces of oxygen in the assay mixture. In other experiments it was shown that reactions lacking β-hydroxybutyrate could be supported by relatively high concentrations of NADH provided that benzyl viologen was included in the reactions. Obviously any dehydrogenase capable of maintaining a supply of NADH would function as a source of reducing power. The sequence of electron flow from

Table 6. Essential components for β-hydroxybutyrate dependent acetylene reduction (after Klucas et al. [31])

The complete reaction mixture in a final volume of 2 ml contained: an ATP-generating system (75 μmoles of creatine phosphate, 7.5 μmoles of ATP, 10 μmoles of $MgCl_2$, 0.2 mg creatine phosphokinase); 200 μmoles DL-β-hydroxybutyrate, 0.5 μmole of benzylviologen; 1.0 μmole NADH, 120 μmoles Tes buffer at pH 7.5 and 0.2 ml of soybean nodule bacteroid extract containing 6.8 mg of protein. The gas volume (19 ml) was composed of 0.1 atm of acetylene and 0.9 atm of argon.

Reaction	C_2H_4 formed (mμmoles/20 min)
Complete	268
Without ATP system	0
" benzylviologen	0
" NADH	21
" β-hydroxybutyrate	64

substrate to nitrogenase is illustrated in Figure 7. In the experiment just described benzyl viologen functioned in place of the flavoprotein (FP) which is included in the diagram.

In a search for a natural component capable of serving in place of the dye (Fig. 6) Klucas et al. (32) have discovered that a protein factor from an acetone powder of bacteroid cell walls, in conjunction with either FAD or FMN will replace the benzyl viologen that was used initially in the system. The evidence now available suggests that NADH dehydrogenase transfers electrons to nitrogenase via a flavoprotein, however this research is incomplete and some alternate interpretations have not been ruled out. An electron transport sequence similar to that found in nodule bacteroids also has been demonstrated with extracts of *Azotobacter vinelandii* (31).

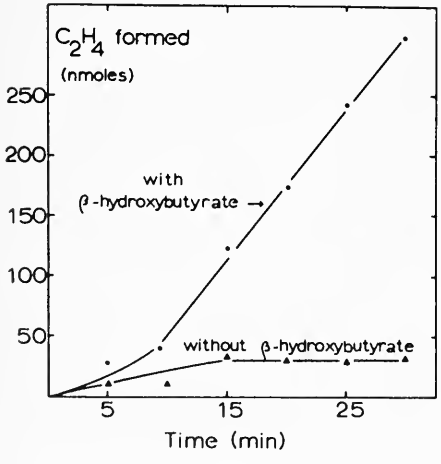


Fig. 6. Time course for the nitrogenase dependent reduction of acetylene in presence of β-hydroxybutyrate (after Klucas and Evans [31]). The reaction with β-hydroxybutyrate contained the same quantities of components as the complete reaction of Table 6.

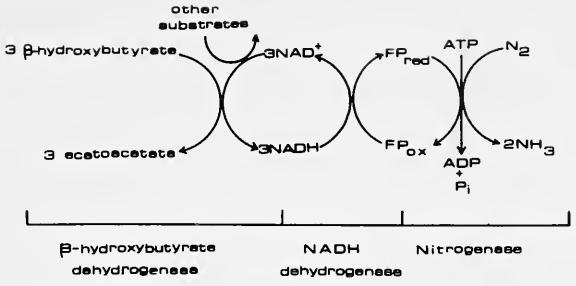


Fig. 7. An electron transport scheme from β-hydroxybutyrate to nitrogenase (after Klucas and Evans [31]).

Conclusions

Considerable progress has been made in recent years in understanding symbiotic nitrogen fixation. Despite the complexities of the symbiosis between *Rhizobium* species and legumes it is now possible to macerate nodules and separate the mixture into a bacteroid preparation, soluble proteins (including the hemoglobin) and plant cellular material and demonstrate with certainty that the bacteroids are the site of nitrogen fixation. Procedures also have been developed for the preparation from bacteroids of purified nitrogenase that exhibits properties similar to those of the nitrogenase preparations from other nitrogen fixing organisms. This was possible after the oxygen lability of nitrogenase was realized, and methods for the prevention of inactivation of bacteroid nitrogenase by oxygen and phenolic compounds became available. Furthermore, the discovery of acetylene as one of several substrates for nitrogenase and the application of this finding to the development of a sensitive assay for nitrogenase has contributed greatly toward progress. All this has aided in the design of feasible experiments to elucidate the chain of electron transport from respiratory substrates to nitrogenase.

Many aspects of the mechanism of symbiotic nitrogen fixation remain unclear. For example, the question of the role of nodule hemoglobin has not been answered. Now that the oxygen sensitivity of nodule nitrogenase has been demonstrated, the postulated role of hemoglobin in the transfer of O_2 to respiratory process of the nodule in a unique way that avoids inactivation of the nitrogenase remains attractive. It is well established that reductant, an ATP supply, and fractions I and II proteins of nitrogenase are all essential for N_2 reduction but the precise role of ATP, and the mechanisms whereby non-heme iron and molybdenum participate in the binding and reduction of N_2 or of other nitrogenase substrates remains in the form of hypothetical schemes.

A major question at the very basis of *Rhizobium*-legume symbiosis concerns why bacteroids within nodules of legumes synthesize nitrogenase and vigorously reduce nitrogen within this partially anaerobic en-

vironment, yet *Rhizobium* species in pure cultures behave as obligate aerobes and exhibit no capacity to synthesize nitrogenase regardless of the manipulation of the environmental conditions. In my opinion the basis for the "text book concepts" of the relationship of free-living *Rhizobium* species and nodule bacteroids to the process of symbiotic nitrogen fixation may require a thorough re-examination.

February 17, 1969

REFERENCES

1. Abel, K., *Phytochemistry,* 2: 429-435, (1963).
2. Allen, Ethel K., and Allen, O. N., *Encyclopedia of Plant Physiology,* Vol. VIII, p. 48-118. Ed. W. Ruhland, Springer Verlag, (1958).
3. Aprison, M. H., McGee, N. E. and Burris, R. H., *J. Biol. Chem.,* 208: 29-39, (1954).
4. Atwater, W. O., *Am. Chem. J.,* 6: 365-388, (1885).
5. Beijerinck, M. W., *Bot. Ztg.,* 46: 725-757, (1888).
6. Bergersen, F. J., *Bact. Rev.,* 24: 246, (1960).
7. Bergersen, F. J., *Biochim. biophys. Acta.,* 130: 304-312, (1967).
8. Bergersen, F. J. and Turner, G. L., *Biochim. biophys. Acta.,* 141: 507-515, (1967).
9. Bergersen, F. J. and Turner, G. L., *J. gen. Microbiol.,* 53: 205-220, (1968).
10. Bergersen, F. J. and Wilson, P. W., *Proc. Natl. Acad. Sci.,* U.S., 45: 1641-1646, (1959).
11. Bond, G., *Symbiotic Associations,* p. 72-91, Eds., P. S. Nutman and Barbara Mosse, Cambridge University Press, (1963).
12. Bowen, H. J. M., *Trace Elements in Biochemistry,* p. 47, Academic Press, New York, (1966).
13. Boussingault, J. B., *Ann. Chim. Phys.,* 2: Ser. 67, 5, (1838).
14. Bulen, W. A., Burns, R. C., and LeComte, J. R., *Proc. Natl. Acad. Sci.,* 53: 532-539, (1965).
15. Burris, R. H., *Inorganic Nitrogen Metabolism,* Ed. W. D. McElroy and Bently Glass, p. 316, The Johns Hopkins University Press, Baltimore, (1956).
16. Burris, R. H., *Plant Biochemistry,* Eds., J. Bonner and J. E. Varner, p. 961-979, Academic Press, New York, (1965).
17. Burris, R. H., *Ann. Rev. Plant Physiol.,* 153-183, (1966).
18. Carnahan, J. E., Mortenson, L. E., Mower, H. F., Castle, J. E., *Biochim. biophys. Acta.,* 38: 188-189, (1960).
19. Dilworth, M. J., *Biochim. biophys. Acta.,* 127: 285-294, (1966).
20. Donald, C. M., *Jour. Austr. Inst. agric. Sci.,* 26: 319, (1960).
21. Hardy, R. W. F., and Burns, R. C., *Ann. Rev. Biochem.,* 37: 331-358 (1968).
22. Hardy, R. W. F., Holsten, R. D., Jackson, E. K. and Burns, R. C., *Plant Physiol.,* 43: 1185-1208, (1968).
23. Hardy, R. W. F., and Knight, E., Jr., *Biochim. biophys. Acta.,* 139, 69, (1967).
24. Hayward, A. C., Forsyth, C. C., Roberts, J. B., *J. gen. Microbiol.,* 20: 510-518, (1959).
25. Hellriegel, H., and Wilfarth, H., *Beilageheft Z. ver Rübenzucker Industrie deut. Reichs.,* (1888).
26. Hoch, G. E., Little, H. N., Burris, R. H., *Nature,* 179: 430-431, (1957).
27. Hoch, G. E., Schneider, K. C., and Burris, R. H., *Biochim. biophys. Acta.,* 37: 273, (1960).
28. Johnson, Samuel W., *How Crops Feed.* Orange Judd & Co., N. Y., (1870).
29. Kennedy, I. R., *Biochim. biophys. Acta.,* 130: 517-519, (1966).
30. Klucas, R., and Burris, R. H., *Biochim. biophys. Acta.,* 136: 399-401, (1967).
31. Klucas, R. V., and Evans, H. J., *Plant Physiol.,* 43: 1458-1460, (1968).

32. Klucas, R. V., and Evans, H. J., (unpublished research).
33. Klucas, R. V., Koch, B., Russell, S. A., and Evans, H. J., *Plant Physiol.*, 43: 1906-1912 (1968).
34. Koch, B. L., and Evans, H. J., *Plant Physiol.*, 41: 1748-1750, (1966).
35. Koch, B., Evans, H. J., and Russell, S., *Plant Physiol.*, 42: 466-468, (1967).
36. Koch, B., Evans, H. J., and Russell, S., *Proc. Natl. Acad. Sci.*, 58: 1343-1350, (1967).
37. Kubo, H., *Acta. phytochim. (Japan)*, 11: 195-200, (1939).
38. Loomis, W. D., and Battaile, J., *Phytochem.*, 5: 423-438, (1966).
39. Nutman, P. S., *Symbiotic Associations*, Ed., P. Nutman and Barbara Mosse, p. 51-69, Cambridge University Press, (1963).
40. Schöllhorn, R., and Burris, R. H., *Fed. Proc.*, pt. II. 25: 710, (1966).
41. Schöllhorn, R., and Burris, R. H., *Proc. Natl. Acad. Sci.*, 58: 213-216, (1967).
42. Schloesing, T., and Laurent, E., *C. R. Acad. Sci.*, Paris III, 750, (1890).
43. Smith, J. D., *Biochem. J.*, 44: 585-591, (1949).
44. Stewart, W. D. P., Fitzgerald, G. P., and Burris, R. H., *Proc. Natl. Acad. Sci.*, U.S., 58: 2071, (1967).
45. Thornton, H. G., *Sci. Progr.*, 31: 236-249, (1936).
46. Virtanen, A. I., and Miettinen, J. K., *Plant Physiol.*, Vol. III, *Inorganic Nutrition of Plants*, Ed. F. C. Steward, Academic Press, New York, p. 539-668, (1963).
47. Wilson, P. W., Biological Nitrogen Fixation—Early American Style. *Bacteriol. Rev.*, 27: 405-416, (1963).
48. Wilson, P. W., Umbreit, W. W., and Lee, S. B., *Biochem. J.*, 32: 2084-2095, (1938).
49. Wilson, P. W., Umbreit, W. W., and Lee, S. B., *Arch. Mikrobiol.*, 8: 440-457, (1937).

How Bacteria Damage Crops

Arthur Kelman

Approximately 50,000 parasitic and nonparasitic diseases affecting species in over 1,200 genera of plants have been recorded in the Handbook of Plant Diseases in the United States (USDA, 1960). These diseases encompass a tremendous diversity of causal agents of disease and the complete range of taxonomic types in the higher plants. The total number of diseases recorded is still a fraction of the potential total. Only a small percentage of the total number of fungi, bacteria, and nematodes that have been described can attack plants. Furthermore, most plant pathogens including parasitic seed plants have relatively restricted host ranges. In addition, although all known viruses may be considered pathogens, viruses affecting animals, other than insects, do not typically attack plants. As yet little is known as to the host range of mycoplasma-like organisms that have recently been reported as plant pathogens. Thus, in directing our attention to the ways in which plants succumb to disease, we must marvel at the remarkable resilience of plants to attack and emphasize that disease is a rare event in terms of the potential threat that exists.

In recent years there has been a great expansion of research in those aspects of plant pathology concerned with the ways in which plants respond to infection and the ways in which pathogens elicit these responses. The tools of the biochemist, the physiologist, the geneticist, and the molecular biologist are now being used more intensively to answer the basic questions: "How do crops sicken?" and "How do pathogens induce disease?" The titles of recent international conferences indicate the expanding research in these areas: "Host-Parasite Relations in Plant Pathology" in Hungary in 1964; "The Dynamic Role of Molecular Constituents in Plant-Disease Interactions" in Japan in 1966; "Biochemical Regulation in Diseased Plants or Injury" in Japan in 1967; and "Physiological and Biochemical Aspects of Host-Pathogen Interactions" in the Netherlands in 1968.

Several recent textbooks survey this area of pathology including: "Biochemistry and Physiology of Plant Immunity" (Rubin and Artsikovskaya, 1963); "The Biochemistry and Physiology of Infectious Plant Disease" (Goodman, et al, 1967); "Physiological Plant Pathology" (Wood,

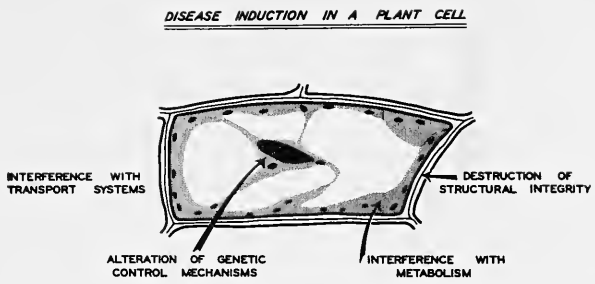


Fig. 1

1967); and Volumes 1 and 2 of the Advanced Treatise on Plant Pathology by Horsfall and Dimond (1959, 1960). Many review articles have appeared in Annual Review of Phytopathology and other texts or journals (Bollard and Matthews, 1966; Yarwood, 1967).

A susceptible plant must have two general characteristics. First it must have a certain degree of compatibility with the pathogen. In initial interaction with a parasite, it must tolerate to some degree the intrusion of the foreign protoplast and permit the invader to grow. Nonpathogenic or incompatible organisms either elicit a rapid reaction (hypersensitivity response) and die rapidly or else simply do not initiate growth (Klement and Goodman, 1967). Secondly, the host must not inhibit or restrict those aggressive mechanisms of the pathogen that enable it to obtain food and to spread through the host tissue.

In general, interference with one or more essential physiological functions or processes of a susceptible plant results in disease (Figure 1). The processes vulnerable to interference by pathogens are interdependent. The disruption or alteration of one or more of the following major processes results in disease (McNew, 1960). (1) *Prevention of seedling development and germination.* The destruction of food reserves and the attack on juvenile tissues characterizes a group of pathogens that may be seed-borne or mainly soil-borne resulting in death of the young seedling before photosynthesis has provided new energy sources. (2) *Interference with root development and uptake of water and mineral nutrients.* A large number of soil-borne organisms including the highly specialized plant parasitic nematodes, soil-inhabiting fungi, and a few bacteria may be involved in the diseases that destroy or attack the developing root system. (3) *Interference with water transport.* The vascular parasites that are involved in the disruption of water movement represent a unique and intriguing group of bacteria and fungi. Initially almost entirely restricted to dead cells of the xylem they bring about profound changes resulting mainly in restriction of water movement, but indirectly affecting many other processes in the host. (4) *Interference with translocation of food reserves.* Viruses, specialized parasitic seed plants, as well as canker and tumor-inducing fungi and

bacteria are associated with this type of disease. The total amount of food being translocated from photosynthetic areas to the roots is reduced. As a secondary effect root development is impaired and the root system is predisposed to invasion by soil-borne organisms. (5) *Reduction in photosynthesis.* A multitude of foliage pathogens affect the photosynthetic processes, directly by decreasing total leaf area and indirectly by altering metabolic activities in the leaves with adverse effects on photosynthesis. Interference with mineral nutrient uptake and water movement affect photosynthesis indirectly. The types of organisms involved range from those causing destructive blights such as fire blight and late blight to complex obligate parasites such as the downy mildews, heterocyclic rusts, and the viruses. (6) *Reproduction.* The group of pathogens, such as the cereal smuts, that directly affect reproductive parts contrast with a large group of fungi and bacteria that are relatively nonspecialized pathogens degrading parenchymatous tissues in developing fruits and seeds. (7) *Destruction of storage food reserves.* A group of relatively weak parasites or pathogens, many of which live as saprophytes, invade the parenchymatous storage organs and fruits of host plants. Usually they are organisms that require wounds or some other injury in order to invade suscept tissue.

In considering the ways in which essential functions of the host are altered in disease, we gain no real insight into the disease process unless we also examine the changes going on at the cellular level. It is possible to divide these cellular changes into four major categories. In any given disease one or more of these cellular effects are involved (Figure 2).

(1) *Destruction of structural integrity.* Although hydrolytic enzyme systems capable of attacking cell wall polysaccharides appear to be essential for pathogenesis, the relative importance of cell wall degrading enzymes for different pathogens is certainly variable (Husain and Kelman, 1959; Wood, 1960). Pectic enzymes alone have been considered to be the sole factors necessary for tissue degradation. It appears now that one or more of several different enzymatic reactions may be involved to some degree in tissue maceration (Figure 3). Only recently have attempts been made to distinguish between the characteristics of enzymes formed in the host and those formed in culture (Bateman and Millar, 1966; Bateman, 1968). On the basis of recent attempts to purify and differentiate pectic enzymes and other hydrolytic systems it is apparent that early investigators were using ill-defined mixtures of one or more enzymes. As a result many publications on the nature and action of pectic enzymes are extremely difficult to interpret. For certain host-pathogen interactions ability of the parasite to secrete cell wall degrading enzymes may be the basis for pathogenicity and host specificity. Enzymes that can hydrolyze the cell wall polysaccharides are characterized by a high degree of specificity. Induction and regulation of enzyme synthesis during the critical period of initial invasion may

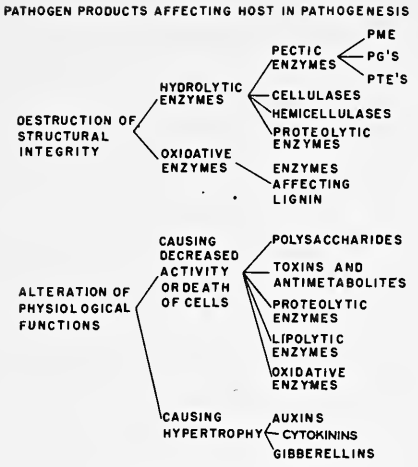


Fig. 2.

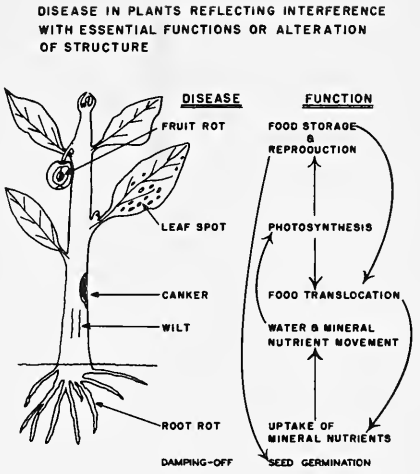


Fig. 3

provide a means of determining susceptible or resistant reactions (Albersheim, et al., 1968).

(2) *Interference with transport systems.* This effect may result indirectly from the action of xylem or phloem invaders or it may reflect deposition of compounds that block movement of essential nutrients into the cell. Competition for nutrients and water in adjacent diseased cells may also affect the availability and rate of movement into the cell of compounds essential for normal metabolism (Beckman, 1964; Dimond, 1955, 1967).

(3) *Interference with metabolism.* A major group of diseases reflect alterations in the metabolism of the cell. These effects may be of two main types. (a) Toxic compounds, produced by the parasite or by the host under stimulus by the parasite, may block essential enzymatic reactions, interfere with protein synthesis, and generally disrupt normal metabolic processes or physiological functions. The types of compounds that have been implicated in these injurious effects include polysaccharides, toxins and antimetabolites, and proteolytic, lipolytic and oxidative enzymes (Figure 3) (Dimond and Waggoner, 1953; Ludwig, 1960; Pringle and Scheffer, 1964; Scheffer and Pringle, 1967; Wheeler and Luke, 1963). (b) Growth substances such as indoleacetic acid, cytokinins, gibberellins, or ethylene may increase in the host stimulating profound and complex changes in the metabolism and growth of the cell (Burg, 1962; Helgeson, 1968; Sequeira, 1963).

(4) *Alteration of genetic control mechanisms.* An elite group of highly specialized pathogens interfere with the expression of the genetic control

system of the cell itself. Viruses (Bawden, 1964; Diener, 1963), certain obligate fungal parasites and the crown gall bacterium (Braun, 1962) are involved in diseases of this type.

Marked changes in patterns of metabolism occur in the cell. These do not reflect changes in genetic material of the cell, but an alteration in the mode of expression of genetic information.

In diseases involving interference with metabolism or alteration of genetic control mechanisms at the cellular level, symptoms may reflect biochemical events in which the metabolites of the host play a more important role than those of the pathogen (Allen, 1966). The ability of viruses to elicit symptoms of abnormal growth or of necrosis supports the concept that symptoms of many diseases reflect the primary involvement of biochemical and physiological processes of the host rather than the pathogen (Diener, 1963). Virus RNA is apparently capable of initiating a series of changes in host cells that result in symptoms typical for a given virus. Furthermore, noninfectious agents can elicit symptoms in plants that may be indistinguishable from those induced by viruses, fungi, bacteria or nematodes.

With this concept in view it would appear that an understanding of the disease process in general can be gained by examining the diseases caused by one single group of pathogens, the phytopathogenic bacteria.

Nature of Diseases Induced by Phytopathogenic Bacteria

Among the approximately 150-175 species of phytopathogenic bacteria there are some that can induce the complete range of symptoms characterizing infections caused by other agents of disease (Dowson, 1957; Ercolani, 1968; Stapp, 1961).

Illustrating this diversity in symptoms, examples of the major types of bacterial diseases are listed in Table 1. Most bacterial pathogens induce prenecrotic symptoms such as yellowing and only a small number cause wilting. The majority of bacterial diseases result in cell and tissue death. Relatively few result in hypertrophy, but of these the most intriguing is crown gall. This is the only plant disease characterized by autonomous growth of affected tissue. In each of the diseases listed one or more essential physiological functions (Figure 1) may be affected adversely and one or more of the effects at the cellular level (Figure 2) may be induced.

It is true that phytopathogenic bacteria are not as fastidious or as sophisticated in trapping energy from their hosts as obligate parasites among the fungi and nematodes. Few bacteria can be considered in the category of the viruses with their unique ability to alter the synthetic systems of the host. A reasonably complete spectrum of mechanisms of disease induction is encompassed in the major types of bacterial diseases listed in Table 1 and even in one disease, the bacterial wilt caused by *Pseudomonas solanacearum*.

Table 1. Major types of bacterial diseases based on symptoms

Symptom type	Common name of disease	Scientific name of host	Scientific name of causal agent
Pre-necrotic			
Yellowing	Bacterial wilt of alfalfa	*Medicago sativa* L.	*Corynebacterium insidiosum*
Wilting	Bacterial wilt of solanaceous, musaceous, and other hosts	Wide host range	*Pseudomonas solanacearum*
	Bacterial wilt of cucumber and other cucurbits	*Cucumis sativus* L.	*Erwinia tracheiphila*
Necrotic			
Blight	Fire blight of pear and apple	*Pyrus communis* L. and *Malus sylvestris* Mill.	*Erwinia amylovora*
	Bacterial leaf blight of rice	*Oryza sativa* L.	*Xanthomonas oryzae*
Spot	Wildfire of tobacco	*Nicotiana tabacum* L.	*Pseudomonas tabaci*
	Angular leaf spot of cotton	*Gossypium hirsutum* L.	*Xanthomonas malvacearum*
	Halo blight of beans	*Phaseolus vulgaris* L.	*Pseudomonas phaseolicola*
Rot	Soft rot of vegetables	Wide host range	*Erwinia carotovora*
Canker	Bacterial canker of stone fruits	Wide host range	*Pseudomonas syringae*
	Bacterial canker of tomato	*Lycopersicon esculentum* Mill.	*Corynebacterium michiganense*
Hypertrophic			
Gall	Crown gall	Wide host range	*Agrobacterium tumefaciens*
Fasciation	Leafy gall of sweet pea and other hosts	*Lathyrus odoratus* L.	*Corynebacterium fascians*
Scab	Potato scab	*Solanum tuberosum* L.	*Streptomyces scabies*

Certain changes in host cells during early stages of pathogenesis characterize all diseases caused by bacteria. These include a rise in respiration, accumulation of phenols, an increase in cell permeability, and an increase in enzymes with peroxidative activity. It should be emphasized that other plant pathogens also elicit similar patterns of change (Goodman, et al., 1967).

Increases in certain isoenzymes of peroxidase have been attributed to *de novo* synthesis. Pathogenic fungi and bacteria, certain viruses,

wounding and ethylene have also been shown to stimulate synthesis of peroxidase and polyphenol oxidase (Farkas and Kiraly, 1962; Kuc, 1967; Stahmann, 1967). Isoenzymes of both host and pathogen may be involved.

Although relatively few investigators have tried to determine permeability changes in bacterial diseases (Wheeler and Hanchey, 1968), symptoms and other evidence support the generalization that increase in membrane permeability occurs in bacterial infections in general and the hypersensitive reaction in particular (Goodman, et al., 1967; Goodman, 1968).

One of the more exciting recent developments in studies on host reactions to bacterial infections is the clear demonstration of the hypersensitive reaction (Klement and Goodman, 1967) following introduction of incompatible bacterial pathogens into specific test plants such as bean or tobacco.

Evaluation of the specific nature of the hypersensitive reaction indicates that toxins, growth substances, or cell wall degrading enzymes may not be involved directly in this phenomenon. It appears that those products or properties of the bacterial cell essential for pathogenicity may be distinguished from the extracellular products such as hydrolytic enzymes normally considered essential in disease development. Many phytopathogenic bacteria can multiply initially in nonhost plants. This capability has resulted in erroneous observations by some investigators exploring the host ranges of bacterial plant pathogens.

The exact nature of the inducer system that triggers the violent reaction of the host in a hypersensitive response has not been determined. Intensive study of this phenomenon may provide an understanding of some of the key determinants of pathogenicity, since many phytopathogenic bacteria possess the inducer, but saprophytic bacteria do not.

The following discussion considers the mechanisms of pathogenesis for bacteria in general and for *P. solanacearum*. This pathogen exemplifies those bacteria that elicit a range of diverse effects in their host plants.

Interference with Transport Systems

The mechanisms of wilting have been studied in depth for certain vascular parasites; in particular, the Fusarium and Verticillium wilt fungi (Beckman, 1964; Dimond, 1955, 1967). In contrast, relatively few studies in depth have attempted to determine exactly how bacteria cause plants to wilt. The mechanical plugging theory, first suggested by Stewart in 1897 for bacterial wilt of corn (*Zea mays* L.), was merely based on the observation that many of the vessels of host plants infected with *Xanthomonas stewarti* were filled with masses of cells. Wilting was considered to result from an interference with the water supply caused by growth of the organism within the vessels and to a lesser

extent by the formation of gums and tyloses by the host in response to invasion. Experimental evidence in support of this or other theories was not provided in most studies.

Perhaps the first report attributing wilting in plants to the formation by a vascular parasite of a systemic toxin was presented in 1913 by Hutchinson based on his studies on *P. solanacearum* (Kelman, 1953). This attractive hypothesis supported and expanded by many other investigators dominated research emphasis for many years in studies on wilting mechanisms for other vascular diseases, particularly Fusarium wilt (Gäumann, 1951). The concept that wilting is attributable to the disruption of osmotic controls by a toxin resulting in excessive transpiration in the leaves is no longer tenable in the light of recent studies on the nature of wilt induction for fungal pathogens (Dimond, 1967). Decreased rate of water flow in the xylem may result from direct and indirect effects of the products of the pathogen or substances formed by the host in response to infection (see Figure 4).

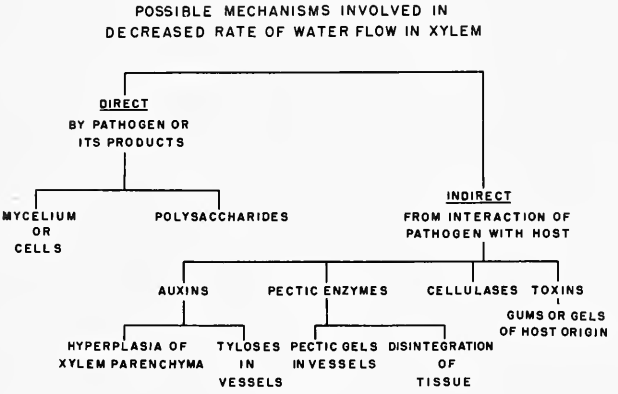


Fig. 4

Experimental evidence to support the mechanical plugging concept for bacteria was provided by Harris (1940) in studies on wilt induction by *X. stewarti* and *Erwinia tracheiphila.* Autoclaved culture filtrates brought about wilting as readily as nonautoclaved indicating the presence of a heat stable nonenzymatic, wilt inducing substance. Measurements of water loss and rate of movement of water in stems of inoculated and noninoculated plants supported the concept that wilting was attributable to an interference with water movement in the stems. Additional support for the plugging concept resulting from formation of extracellular polysaccharides by xylem-invading bacteria has been provided by Sutton and Williams (1968) for *Xanthomonas campestris* and by Spencer and Gorin (1961) in studies with *Corynebacterium insidiosum*

and *C. sepedonicum*. This aspect of wilt induction is discussed in more detail below for *P. solanacearum*. Strobel and Hess (1968) and Rai and Strobel (1969), however, question the conclusion that vascular plugging causes wilting in alfalfa infected by *C. sepedonicum* and tomato infected by *C. michiganense*.

Wilt-inducing polysaccharides, both extracellular and intracellular in origin, have been obtained from a number of bacteria that have no wilt-inducing capacity in their host plants (Leach, et al., 1957; Starr, 1959). Thus, one may question the relevance to pathogenesis of demonstrations of wilt-induction by culture filtrates of bacteria that neither invade xylem tissue nor induce wilting as part of the disease syndrome.

The exact role of the polysaccharide in those diseases in which the pathogen invades parenchymatous tissue causing rots, leaf spots, cankers, and blights is still quite obscure. In all of these diseases cells outside of the lesions and parts far removed from the infection usually are deprived of nutrients and water.

A phytotoxic glycopeptide produced by *Corynebacterium sepedonicum* induced wilting of plant cuttings at relatively low concentrations (Strobel and Hess, 1968). The glycopeptide apparently destroys the integrity of cellular membranes resulting in a net loss of water from parenchymatous cells. Damage attributed to the glycopeptide was observed in chloroplasts, mitochondria, and plasma membranes. The damage appeared to be a direct effect and not a secondary result of the action of the glycopeptide. Furthermore, the cytological abnormalities were thought to be due to the intact toxic compound and not a breakdown product. Water movement was as rapid through glycopeptide-treated stems as nontreated stems, although high molecular weight polysaccharides greatly retarded dye movements in other plants tested. If membrane permeability is a factor in the mechanism of action of the glycopeptide, then the ability of treated cells to plasmolyze may be affected. In support of this concept it was found that glycopeptide-treated cells did not plasmolyze and furthermore that electrolyte loss was greater from treated cuttings than from the controls. The water imbalance attributable to the action of these toxins may result in wilt induction.

Destruction of Structural Integrity

Tissue degradation is a dominant feature of many major diseases and the ability to penetrate cell walls appears to be an essential trait of most plant pathogens (Husain and Kelman, 1959; Bateman and Millar, 1966). Strong evidence has been presented to implicate one or more of the multitude of pectic enzymes as the primary or sole factor in tissue maceration. However, *in vivo* hemicellulases, phosphatidases, and proteases as well as cellulases may be involved to some degree.

In bacterial soft rot diseases the initiation of cell wall degradation

precedes the death of the protoplasts. Specific toxic substances produced by the pathogen have not been identified nor have such compounds been implicated in the death of cells. Proteolytic enzymes are actively produced by soft rot bacteria and presumably could be involved in loss in permeability of membranes or membrane degradation. The phosphatidases have only recently been examined with respect to their role in tissue maceration. Apparently soft rot bacteria do produce large amounts of phosphatidase and an examination should be made of these enzymes for their specific role in mechanisms of tissue degradation (Tseng and Bateman, 1968). Furthermore, compounds released by cell wall or protoplast degradation may in themselves be toxic.

In most studies on tissue degradation primary emphasis has been placed on the pectic enzymes of soft rot bacteria. There is a need to consider the relative importance of macerating enzymes produced by the host. In the case of soft rot diseases host formed cell wall degrading systems may be activated to a higher degree than has been realized. This has recently been explored by Tani (1967) in studies on the softening of kaki fruit (*Diospyros kaki* L.) following infection by the anthracnose fungus, *Gloeosporium kaki* Hori. Tani concluded that maceration of the fruit was more attributable to the action of cell wall degrading enzymes formed by the host than the pathogen. Similar observations with soft rot bacteria have not been made, however. Comparisons of healthy and diseased tissues may also be deceptive since enzyme induction by the host may not occur until infection has been established.

Cell bound dehydrogenase of *Erwinia carotovora* may be involved indirectly in the degradation of host tissue (Lovrekovich, et al., 1967). The oxidation products of polyphenol oxidase in potato are associated with limitation of spread of the soft rot bacteria in potato tuber tissue. Bacterial cell suspensions were able to prevent darkening of potato sap and to reverse catechol oxidation by polyphenol oxidase in the presence of glucose. Maintenance of a reduced state is apparently an essential factor in the process of maceration. Oxidation of phenolics released by tissue maceration results in inhibition of the rotting process. Formation of oxidation products is inhibited by the dehydrogenase system of the soft rot pathogen. In the absence of such systems the bacterial soft rot organisms presumably could not continue to develop in tuber tissue.

This concept of localization of decay organisms differs from that propounded by Bateman in studies with a fungal pathogen, *Rhizoctonia* (Bateman and Millar, 1966; Bateman, 1968). Apparently deposition of calcium occurs in cell walls adjacent to the infected area and calcium increases the resistance of pectic substances to hydrolysis by the polygalacturonase of the fungus. Since pectin transeliminase is the major enzyme associated with tissue degradation by soft rot bacteria and the activity of this enzyme is increased in the presence of Ca, increased

availability of calcium could increase maceration of tissue by bacterial soft rot pathogens (Dean and Wood, 1968).

Until relatively recently phytopathogenic bacteria were considered to be unable to degrade cellulose. In a survey of 28 species and 5 genera of phytopathogenic bacteria for cellulase (Cx) formation (Goto and Okabe, 1959a, 1960), Cx was found to be produced by *Xanthomonas* species and all strains of *E. carotovora.* In general, however, levels of Cx formed by *Pseudomonas* species, with the exception of *P. solanacearum,* were low. Gehring (1962) compared levels of pectic and cellulolytic enzymes formed by typical soft rot bacteria and the two pathogens that cause wilt in carnation (*Dianthus caryophyllus* L.), *Erwinia parthenii* var. *dianthicola* and *Pseudomonas caryophylli.* The *Erwinia* pathogenic on carnations formed lower levels of pectin methyl esterase (PME) and polygalacturonase (PG), but higher levels of cellulase (Cx) than the soft rot *Erwinia* isolates tested. *P. caryophylli* formed cellulase but no detectable PME or PG. Typical soft rot pathogens did not multiply in vascular tissue of carnations and were avirulent on this host. Cellulolytic enzymes are presumably more important than pectic enzymes for invasion of xylem tissue. It is always attractive to postulate a causal relationship between formation or specific hydrolytic enzymes and pathogenic potential, and soft rot bacteria provide good model systems. Even so the exact determinants of host specificity and virulence are still unresolved even in these relatively simple host-pathogen systems.

In consideration of the role of cellulases in tissue degradation it is important to emphasize the need to determine whether or not native cellulose can be degraded in the hosts studied. Relatively few attempts have been made to examine changes in cellulose in the host. However, in general it would appear that cellulase is not as important in tissue maceration as pectic enzymes.

The causal agent of bacterial spot of cucumber, *Pseudomonas lachrymans,* forms a compound in culture that can elicit the symptom of watersoaking (Keen, et al., 1967a,b, 1969). Originally it was thought that this was a proteolytic enzyme. Subsequently it was shown that the assay used for protease actually reflected a noncatalytic change in the solubility of the protein in trichloroacetic acid (TCA). A fraction containing a lipomucopolysaccharide from cells produced TCA-soluble material when incubated with casein and other proteins, but it did not exhibit peptidase activity, decrease viscosity of gelatin, or hydrolyze various synthetic substrates hydrolyzed by proteases such as trypsin or papain. Some proteolytic activity could be demonstrated as distinct from the compound affecting protein-solubility. Further studies are needed to determine the exact nature of the extracellular products of this pathogen and the specific role that the lipomucopolysaccharide and protease are playing in the development of symptoms; in particular, water-soaking.

Interference with Metabolism by Toxic Compounds

Since most plant disease symptoms reflect a complex and dynamic interplay of metabolites of host and pathogen, it is extremely difficult to demonstrate conclusively how specific metabolites such as toxins can elicit distinctive disease symptoms. In plant pathology, the word toxin has been used in a manner that is not equivalent to usage in medical pathology. Normally a toxin is considered to be a substance of low molecular weight that injures plant cells and/or disrupts their metabolism at relatively low concentrations.

Extracellular polysaccharides produced by bacteria may have two roles in disease induction: (1) physical obstruction of xylem vessels and (2) direct toxic effects on the protoplast. If they play the latter role, then they may be considered in the category of general plant toxins. The toxic glycopeptides of *C. michiganense* and *C. sepedonicum* apparently play the latter role according to Strobel and Hess (1968) (see section on "Alteration of transport mechanisms").

In recent years the structures of a number of toxins formed by fungus pathogens have been studied and modes of action have been postulated (Ludwig, 1960; Scheffer and Pringle, 1967; Wheeler and Luke, 1963). At the present time no host specific toxin similar to that formed by *Helminthosporium victoriae* (Scheffer and Pringle, 1967; Wheeler and Luke, 1963) has been described for plant pathogenic bacteria. Wildfire disease of tobacco caused by *Pseudomonas tabaci* is often cited as one of the few diseases in which the formation of a key symptom can be attributed to a specific toxic substance synthesized by the pathogen (Braun, 1955). The formation of a chlorotic yellow halo around a necrotic spot is the characteristic symptom of this disease on tobacco leaves. It is possible to isolate a toxin from cultures of the bacterium capable of inducing chlorosis not only in tobacco but also in many other plant species, including plants not susceptible to the pathogen. Characterization of the toxin revealed it was a structural analogue of methionine. Experiments involving methionine sulfoximine (MSO) provided support for the theory that the toxin was an antimetabolite of methionine. MSO produced chlorotic halos when injected into tobacco leaves and inhibited growth of the alga Chlorella in a manner very similar to that of the wildfire toxin. This research focused attention on the prospects of explaining disease phenomena on the basis of specific toxic metabolites of plant pathogens. It was recognized at the completion of these studies that there were certain aspects of the problem that were not fully explained, however. One of these was the inability of methionine to prevent injury to tobacco leaves by the wildfire toxin and MSO.

Recent studies by Sinden and Durbin (1968) indicate that both wildfire toxin and MSO inhibit glutamine synthetase, which catalyzes formation of glutamine from glutamic acid and ammonia. Methionine

neither inhibits glutamine synthetase nor protects the enzyme against MSO inhibition. Sinden and Durbin were able to relate *in vitro* enzyme inhibition to symptom induction in tobacco. Concentrations of wildfire toxin that inhibit glutamine synthetase *in vitro* cause chlorosis of tobacco leaves. Treated leaves also were characterized by an increase in ammonia levels to approximately 7 times that of tissue in untreated leaves. By injection of high levels of glutamine into the leaves it was possible to prevent both chlorosis and an increase in levels of ammonia. Thus, inhibition of glutamine synthetase may be the primary action of the tobacco wildfire toxin. Furthermore, the chlorosis is more likely caused by toxic levels of ammonia or other intermediates of nitrate metabolism rather than by an induced deficiency of glutamine. The ammonia presumably arises from continued reduction of nitrate by the plant during a period when the major pathway for ammonia incorporation into organic compounds is not operative. The toxin of *P. coronafaciens* is essentially similar to the exotoxin of *P. tabaci.* Halos produced by *P. phaseolicola* and *P. coronafaciens* were identical and distinguished by distinct margins and more rapid development. Toxins produced by other halo-forming bacteria, *P. phaseolicola, P. glycinea* and *P. tomato,* however, differed from the toxin of *P. tabaci* and *P. coronafaciens.* These toxins were heat-stable and stimulated an increase in ornithine content. The type of chlorotic halo produced was also different.

One of the changes that has been associated with effects of wildfire toxin on host tissues is an increase in peroxidase. Lovrekovich, et al. (1968) have observed that this increase can also be elicited by injection not only of dead cells of *P. tabaci* but also by a substance isolated from *P. tabaci* cells by procedures used to extract endotoxin from other bacteria. The activation of the peroxidase system could be correlated with an increase in resistance in tobacco leaves to infection by the wildfire pathogen. Injection of a commercial peroxidase also retarded symptom development. The phenomenon of induced resistance following injection of heat-killed cells and its correlation with increased peroxidase should be an area of exciting and productive additional investigation.

Studies on the amino acid metabolism of bean leaf tissue infected by *P. phaseolicola* have revealed dramatic shifts in certain amino acids and amides (Patel and Walker, 1963). Histidine, methionine, and ornithine increased markedly. In particular, 20 times as much ornithine was present in chlorotic tissue as in healthy tissue. These changes were attributed to the action of the toxin since bacteria are not usually present in the chlorotic zone. The decrease in protein synthesis observed in infected leaves may be another manifestation of the alteration of amino acid metabolism (Rudolph and Stahmann, 1964).

Recently another bacterial toxin that is produced by a species of the legume root nodule bacterium *Rhizobium* has been studied (Owens, et al., 1968). Although *Rhizobium* is not normally considered to be a pathogen, chlorosis in certain soybean (*Glycine max* L.) varieties is

caused by certain strains of *Rhizobium japonicum.* These strains fix nitrogen in an apparently normal fashion, but at the same time synthesize a toxin that induces chlorosis in the new leaves of soybean plants. The toxin has been isolated from nodules of diseased plants showing chlorosis and also from the culture medium of the bacterium. The toxic material, designated as rhizobitoxin, is similar to the wildfire toxin in that it is not host specific.

At low concentration it may cause chlorosis in seedlings of many different plant species. Growth of *Salmonella typhimurium* was inhibited by low levels of the toxin and inhibition could be completely prevented by simultaneously adding low amounts of methionine or its immediate precursor, homocysteine. Enzymatic cleavage of cystathionine to form homocysteine may be inhibited, thereby inducing a deficiency of methionine. The primary mechanism is apparently the inhibition of cystathionase. Additional studies with plant systems indicate that the inhibition of this enzyme is a major if not the sole mechanism for the action of rhizobitoxin in higher plants. Rhizobitoxin is of interest not only because it is formed by an organism normally considered as mainly beneficial in its interaction with plants, but because it acts in disease induction as an enzyme inhibitor.

Virulent isolates of *Pseudomonas syringae* that cause bacterial canker of peach trees produce a toxin biocidal to many microorganisms and to host tissue (De Vay, et al., 1968). Loss of pathogenicity is correlated with loss in ability to produce the toxin. Partially purified toxin injected into trees elicited symptoms observed in trees inoculated with the pathogen. The antibiotic is a peptide that is produced in host tissues at concentrations high enough to cause symptoms of the disease. The nature of the action of this toxin in the host is not fully understood as yet.

In the case of toxins produced by bacterial pathogens it is important to emphasize that toxigenicity may not always be a prerequisite for pathogenicity. This is true in the case of the halo-forming *Pseudomonas* species since nontoxin-forming strains are capable of infecting host leaves, although chlorotic halos may not be formed.

As we gain more knowledge of the extracellular products of bacterial pathogens, it is likely that many additional toxic compounds will be uncovered.

Interference with Metabolism by Growth Regulators

Growth abnormalities characterize many bacterial diseases. With the exception of crown gall, continued presence of the bacterium is essential for maintaining abnormal growth patterns in these diseases. Among possible factors involved in hypertrophic symptoms for plant diseases in general are (1) alterations in levels of specific growth substances such as IAA, gibberellins, or cytokinins; (2) alterations in levels of enzymes involved in degradation of growth substances; (3) increases in levels of compounds that inhibit action of growth regulators or enzymes that

synthesize or degrade growth substances; (4) decreases of growth substance levels by formation of a complex with other cell metabolites (Sequeira, 1963).

It is beyond the scope of this review to encompass in even a superficial way the wealth of literature on abnormal growth as exemplified by tumor induction by the crown gall bacterium (Braun, 1962; Goodman, et al., 1967; Wood, 1967). The complex and intriguing nature of the crown gall problem has frustrated researchers for half a century. Many studies have claimed to explain the mechanism of tumor induction, but almost invariably it has been shown subsequently that the results were incorrect either because of faulty technique or failure to interpret data properly.

At various times the tumor-inducing principle (TIP) has been considered to be (1) a genetic alteration; (2) a carcinogenic metabolic product of the bacterium; (3) a host constituent converted by the bacterium to a carcinogenic agent; (4) a transforming substance-DNA; (5) a virus introduced by the bacterium; (6) an alteration of the bacterium resulting in its change to a form that cannot be reisolated; (7) a combination of one or more of the preceding types. Recent work has focused attention on the possible introduction of a portion of the bacterial genome into the host DNA.

Two major processes are involved in tumor induction: The first involves conditioning of the host cells following wounding; the second is the induction process in which conditioned cells are converted to autonomous cells by the tumor-inducing principle formed directly or indirectly by the crown gall bacterium. Once the transformation is complete, usually 72 hours after wounding and infection by the bacterium, the cell has acquired the capacity for autonomous growth. The transformed cells are capable of synthesizing cytokinins, gibberellins, and IAA as well as other essential growth requirements.

It is natural that abnormalities in growth of many diseased plants should be attributed to production by the pathogens of abnormal levels of growth regulators. Yet conclusive proof has been lacking that compounds such as IAA or cytokinins are synthesized by organisms *in vivo* at levels high enough to alter growth.

Much circumstantial evidence was presented to support the concept that IAA produced by the crown gall bacterium was a key factor in tumor induction. The separation of the distinct phases of gall production into induction and autonomous growth phases provided a means of discrediting this concept. Clear evidence was obtained indicating that tumor tissue could synthesize levels of IAA and other essential compounds well above those formed in normal cells and once the process was initiated it was independent of the original stimulus. IAA alone could not trigger this autonomous growth.

In recent studies the number of tumors initiated by *A. tumefaciens*

on pinto bean leaves was decreased when cells of an avirulent strain were included in the inoculum (Lippincott and Lippincott, 1969). In addition to living cells and heat-killed cells of the avirulent strain, heat and UV inactivated cells of the virulent strain also inhibited tumor formation. It would appear that tumor induction requires specific complementary binding of a virulent living bacterium to a wound receptor site. Introduction of avirulent cells in the system apparently interferes with the attachment of the virulent cells.

Profound changes occur in the morphology, anatomy, and metabolism of plants treated with ethylene at above-normal physiological levels (Burg, 1962). The exact mechanism of ethylene action and its role in pathogenesis have not been determined. The demonstration of increased levels of ethylene at the site of infection, that certain pathogens produce ethylene or induce its formation by the host and its ability to induce increases in host peroxidase and polyphenoloxidase have stimulated increasing interest in its importance in disease processes in plants (Stahmann, 1967). Although they are difficult, analyses of cause/effect relationships in disease development should receive more attention (Burg, 1962).

One of the very exciting new developments in studies on growth alterations by bacteria was the evidence that a cytokinin produced by *Corynebacterium fascians* could induce disease symptoms in garden pea (*Pisum sativum* L.) (Thimann and Sachs, 1966). This was the first demonstration of the presumptive role that cytokinins may have in growth alterations due to a pathogen. The pattern of outgrowth of lateral buds at a node inoculated with *C. fascians* was reproduced by application of kinetin and also by an extract from the culture filtrate of *C. fascians* containing cytokinin activity. Although only very small quantities of the active ingredient were available, the cytokinin present in the culture was isolated and characterized as 6-(γ,γ-dimethylallylamino) purine (Helgeson, 1968). This compound was found to be 10 times as active as kinetin and only slightly less active than zeatin which is also a 6 substituted purine isolated from corn. One of the distinctive aspects of the work on this compound was the subsequent evidence that it was present as a riboside not only in the tRNA of *C. fascians,* but also in hydrolyzates of tRNA from *Escherichia coli,* yeast, higher plants and mammalian liver tissue. At present it has not been possible to determine the specific role cytokinins play in activating a range of different biochemical events. In reviewing the current status of cytokinin research, Helgeson (1968) emphasized that growth effects elicited by cytokinins reflect complex interactions with IAA and gibberellins as well as other components of the system. It is now evident that another area of research requiring attention in analyses of host-parasite relationships is the determination of the role of cytokinins of plant or parasite origin in the disease process.

Disease Development in Plants Infected by a Wilt-Inducing Pseudomonad

Pseudomonas solanacearum is one of those unique wilt-inducing bacteria that are initially restricted to the xylem elements of their host plants. Although this wilt disease ostensibly reflects a simple host-pathogen relationship, analysis of pathogen products and host responses indicates a relatively complex disease involving a complete range of symptoms (Buddenhagen and Kelman, 1964). The bacterium is soilborne and usually invades the roots through wounds and spreads in the xylem through the roots and into the stem. Under favorable environmental conditions symptoms include wilting, stunting, yellowing, vascular browning and necrosis, epinasty of leaves, and, in some instances, adventitious root formation (Stapp, 1965).

A number of strains differ in specificity to solanaceous (tobacco, tomato, potato, eggplant *Solanum melongena* L.), musaceous (banana *Musa paradisiaca* L. var. *sapientum* Kuntze), and other major economic hosts (peanut *Arachis hypogaea* L.). The bacterium is widely distributed in the warm-temperature, sub-tropical and tropical zones of the world. It ranks as one of the most destructive of all plant pathogens (Kelman, 1953). Formation of a polysaccharide is constantly associated with virulence (Husain and Kelman, 1958). Variants lacking the polysaccharide are either weakly virulent or avirulent and can be readily distinguished from the virulent type by their appearance on solid media containing 2,3,5-triphenyl tetrazolium chloride (Kelman, 1954).

During the past 15 years certain symptoms of the disease syndrome have been related to the action of specific enzymes, other extracellular substances of the pathogen, and host products stimulated by the pathogen-suscept interaction. For each stage in the manifestation of the characteristic pattern of the wilt disease one or more basic physiological processes are affected. In discussing the ways in which plants respond to infection by *P. solanacearum*, emphasis will be placed on aspects of tissue degradation, alterations in metabolism, and wilt induction.

Destruction of Structural Integrity

The possible importance of pectic and cellulolytic enzymes in pathogenesis by wilt-inducing bacteria was overlooked in early studies on this disease. In young tomato or potato plants invaded by *P. solanacearum* cells in the xylem, phloem, and adjacent pith and cortex may be decomposed. In mature plants, where lignification of cell walls may be more advanced, relatively little degradation of xylem occurs. Histologic studies reveal that middle lamellae are degraded in vascular tissue indicating action of pectic enzymes *in vivo* (Husain and Kelman, 1958). Culture filtrates of *Pseudomonas solanacearum* contain relatively high levels of pectin methyl esterase (PME) and also polygalacturonase (PG).

PG and PME formed by the pathogen can be detected in infected

tomato plants. Histologic examination of stems of tomato cuttings placed in culture filtrates containing PME and PG also revealed that pectic substances were degraded. In preliminary studies no pectin-trans-eliminase was detected in culture filtrates even when sodium polypectate was incorporated in the medium. In this respect, *P. solanacearum* differs from the typical soft rot bacteria.

Cellulase (Cx) was detected in extracts of plant stems infected by *P. solanacearum* but not in healthy tissue. The levels found were relatively low, but the method of extraction involved a rather significant dilution. Cell walls of tissues invaded by the bacterium lost the birefringence that was typical in adjacent healthy tissue (Husain and Kelman, 1958) thus affording indirect evidence that cellulose in the cell walls was degraded.

Alpha-cellulose content, the ratio of alpha- to hemicellulose, and the degree of polymerization of holo-cellulose of tomato and tobacco stem tissues all decreased during pathogenesis (Kelman and Cowling, 1965). Chromatographic analyses for cellobiose and glucose in diseased tissues were negative, however. Cellobiose was the main product of hydrolysis of carboxymethylcellulose (CMC) but no glucose was detectable. The isolate tested was unable to utilize cellobiose as a sole carbon source and an active beta-glucosidase was not detected. No reducing substances were formed from purified cellulose preparations obtained from tomato and tobacco when exposed to culture filtrates of *P. solanacearum*. As is true for other bacteria the cellulase of *P. solanacearum* is constitutive since the enzyme was formed on media lacking cellulose or a cellulose derivative. Avirulent mutants of 10 isolates from different hosts and geographic areas produced little or no cellulase (Cx) using two different assay systems.

Although *P. solanacearum* cannot be considered highly cellulolytic in comparison with fungi such as *Trichoderma viride* or *Myrothecium verrucaria* that degrade native cellulose very rapidly, the cellulolytic enzymes of this, and other phytopathogenic bacteria, should not be ignored in developing a full understanding of pathogenesis. The inability of an organism to utilize native cellulose, or some highly purified derivative, as a sole carbon source is not unequivocal evidence that it lacks the ability to degrade cellulose in host tissues. The cellulase of *P. solanacearum* can facilitate penetration of host tissues, although the products of hydrolysis may not be utilized as carbon and energy sources.

Alteration of Metabolism

Changes in respiration rates of tissue from wilt resistant and susceptible tobacco varieties were examined in detail by Maine (1960). Respiration increased in susceptible tissues within two days after infection. Increases in respiration in susceptible and resistant tissue could be correlated with stages of pathogenesis. Polyphenol oxidase activity in-

creased as the symptoms intensified and an early rise of ascorbic acid oxidation also occurred. Increased polyphenoloxidase activity in diseased tissue was attributable to a host response since *P. solanacearum* lacks a polyphenoloxidase. Free amino acids and soluble protein also increased. Aromatic amino acids, particularly L-phenylalanine and L-tryptophan, increased rapidly in young inoculated plants in contrast to levels in healthy controls (Pegg and Sequeira, 1968). A selective stimulation in aromatic amino acid biosynthesis by the plant occurs during a period when the pool of amino acids other than aromatics is decreasing as a result of protein synthesis. These profound alterations in metabolism occur before well developed symptoms of infection are visible. Conversion of phenylalanine to cinnamic acid and then to coumarin-like compounds during this period would contribute to increases in scopoletin. These studies reveal that host reactions are activated even when the pathogen is still localized in the xylem elements.

The reaction of inoculated plants to mixtures of different proportions of virulent and avirulent strains provides additional insight into host reactions to avirulent or incompatible isolates (Averre and Kelman, 1964). Tobacco plants stem-inoculated with mixtures of a high proportion of avirulent to virulent cells (99:1) did not develop wilting symptoms. Similar results were obtained when the mixture contained a high proportion of cells of the banana race (Race 2) that is avirulent on tobacco. In all cases, the number of virulent cells of the tobacco race in the inoculum was high enough to cause rapid infection and wilting when inoculated separately. A post-inoculation host response resulted in localization of the incompatible or avirulent populations. In essence these studies gave evidence of a type of hypersensitive reaction in tobacco stem tissue similar to those reactions that occur when various phytopathogenic bacteria are injected into tobacco leaves (Klement and Goodman, 1967). Lozano and Sequeira (1968) have now demonstrated that the hypersensitivity reaction in tobacco leaves can be used to separate certain races and strains of *P. solanacearum*. The banana race and avirulent isolates of this pathogen induce typical hypersensitivity reactions when infiltrated into tobacco leaves. It appears that *P. solanacearum* may provide a model system for analysis of this reaction using the range of different compatibility types available.

In addition to characteristic wilting symptoms, leaf epinasty and adventitious roots may appear on plants infected by *Pseudomonas solanacearum* (Grieve, 1941b). Studies on auxin imbalance in diseased plants have revealed the interplay of several growth regulating systems (Sequeira and Kelman, 1962; Sequeira, 1963, 1964, 1965, 1969; Pegg and Sequeira, 1968). Following infection, levels of IAA increased greatly in banana and tobacco, particularly in stem tissue invaded by the pathogen. Pathogenic, weakly pathogenic, and avirulent strains of *P. solanacearum* all produced relatively high levels of IAA in culture. When comparisons were made of pathways of synthesis of IAA by

virulent and avirulent strains, it was found that the avirulent produced about 5 times as much IAA as the virulent. Furthermore, the virulent strain preferentially degrades tryptophan through the kynurenine pathway and synthesizes IAA from products of this pathway retaining parts of the original benzene ring. This is a pathway not previously described for bacteria. In contrast to the above, the avirulent strain mainly follows the more common pathway of IAA synthesis from tryptophan by transamination to 3-indolepyruvic acid which is then decarboxylated to 3-indoleacetaldehyde (Phelps and Sequeira, 1967).

Epinasty can be induced in tomato cuttings immersed in solutions containing IAA levels equivalent to those present in the tissues of diseased plants. During the early stages of infection, increases in IAA are mainly attributable to host response rather than to production by the wilt bacterium. Increase in IAA not only reflects synthesis by the host and the pathogen, but also inhibition of the degradation of IAA. IAA oxidase activity, particularly in the roots of tobacco plants, is markedly reduced following infection. Reduction in activity is correlated with increased levels of scopoletin. Scopoletin can inhibit IAA oxidase at concentrations of 1-9 ppm (Sequeira, 1964, 1969). Scopolin (a glucoside of scopoletin) and its aglucone were shown to accumulate in tissues adjoining xylem vessels of tobacco plants invaded by *P. solanacearum* (Sequeira, 1969). In stem tissues sampled 48 hours after inoculation scopolin increased 3 times above that of healthy tissue. Scopoletin increased approximately 24 hours after the rise in scopolin content and reached levels 18 times that of normal tissue by 120 hours after inoculation. Rapid increase in scopoletin was not attributable to increased hydrolysis of scopolin but to synthesis of both during disease development.

One of the distinctive early symptoms of plants inoculated with *P. solanacearum* is the cessation of elongation. In addition to growth-promoting substances, Sequeira and Kelman (1962) observed that concentrations of an inhibitory compound also increased following infection of tobacco and banana plants. Recently Steadman and Sequeira (1969) have investigated the changes in inhibitor concentration associated with disease development. An ether-soluble acidic substance that strongly inhibits wheat coleoptile extension was detected in both healthy and diseased tobacco plants. However, in stem tissues of tobacco plants infected with *P. solanacearum* concentrations of the inhibitor were increased two-fold. The relative increase in concentration of the inhibitor in the stem was correlated with distribution of the bacterium. The compound was highly active causing growth inhibition at levels of 5 μg/ml. The compound has properties similar to the so-called inhibitor-β or abscisic acid. Strong evidence was obtained to support the concept that inhibitor synthesis is a result of a host response. Marked growth inhibition precedes the time that decreased supplies of water and nutrients may affect growth significantly. Thus, the growth inhibitor may play

a key role in early stunting effects. Determination of the exact nature of these effects will be dependent on chemical characterization of the inhibitor.

The first demonstration of formation of ethylene by a bacterial pathogen *in vitro* resulted from studies on *P. solanacearum* (Freebairn and Buddenhagen, 1964). Several different strains produce ethylene at detectable levels. Premature ripening of fruit infected by *P. solanacearum* was associated with high internal levels of ethylene. The time sequence and relative rates of ethylene formation by the host and the pathogen during infection are difficult to resolve. The importance of ethylene in inducing a wide range of host reactions in the critical early stages of pathogenesis has been stressed previously. It is possible that ethylene is also involved in epinasty and other host responses attributed to IAA imbalance in infected plants.

Interference with Transport Mechanisms

Wilt induction in *P. solanacearum* is attributed mainly to interference with water movement in the stem rather than to reduced water uptake by the roots or excessive water loss from the leaves (Grieve, 1941a; Husain and Kelman, 1958). Factors considered to be involved in wilt induction are (1) formation of bacterial polysaccharide or slime and cells in the vessels, (2) degradation of cell wall components by pectic and cellulolytic enzymes, and (3) stimulation by IAA of tylose formation and vessel collapse. Analysis of the factors involved in wilt induction by *P. solanacearum* has been facilitated by the ease of differentiating between virulent and avirulent strains which allows comparisons with both types (Husain and Kelman, 1958).

The extracellular polysaccharide is considered to play a primary role in the wilting mechanism (Husain and Kelman, 1958). The following evidence supports this concept: (1) a heat-stable wilt-inducing viscous substance can be obtained from xylem tissue of infected plants; (2) an extracellular heat-stable wilt-inducing polysaccharide is formed by virulent strains in culture media. Avirulent or weakly virulent strains do not form this compound in significant amounts. Cuttings with xylem vessels infiltrated with high populations of the avirulent strain do not wilt; (3) a solution of a partially purified polysaccharide obtained from the culture filtrates of the virulent strain caused rapid wilting of tomato and tobacco cuttings; (4) the extracellular polysaccharide formed by the virulent strain in culture can also be demonstrated in infected plants; (5) as shown by application of suction to invaded stem segments and recovery of wilted leaves from stem-inoculated plants when placed in water, the efficiency of the water conducting system is drastically reduced during disease development.

Measurements of photosynthesis and transpiration on leaves of inoculated and noninoculated banana plants (Beckman, et al., 1962) indicated that leaves of infected plants have a normal capacity for both.

Toxic effects on the photosynthetic mechanism or the function of guard cells were not detected even under conditions when leaves were in the stage of irreversible wilting. These observations show that vascular occlusion limiting availability of water was the primary factor in wilting rather than a toxin affecting osmotic relations in leaf cells.

Pectic enzymes of the wilt bacterium may not be involved in the wilting process in the manner that has been postulated for those of *Fusarium oxysporum* f. *lycopersici.* Culture filtrates of nonpolysaccharide-forming weakly virulent or avirulent strains of *P. solanacearum* contained PME and PG at relatively high levels but did not induce wilting symptoms in tomato cuttings (Husain and Kelman, 1958). A solution of a highly purified fungal PG also did not cause wilting, although histologic studies showed that the middle lamellae of cells in the vascular tissues were decomposed.

The presence of IAA and possibly ethylene in the xylem elements may also enhance the primary effect of the polysaccharide in occlusion of the vessels. Presumably IAA could stimulate tylose formation and vessel collapse in advance of massive multiplication of the bacterium in the vessels (Sequeira and Kelman, 1962).

Summary

In summarizing the present status of knowledge of changes in the host during pathogenesis by the wilt pathogen *P. solanacearum,* these alterations can be classified under general processes affecting the plant as a whole and specific changes in enzymes or metabolites in affected tissues. The following physiological processes are depressed or decline as the disease progresses: transpiration, uptake of mineral nutrients and water, water and mineral nutrient transport in the xylem, food transport in the phloem, and photosynthesis.

Respiration increases rapidly in invaded tissue. The following metabolites also increase in the tissues adjacent to the infected vascular tissues: scopolin, scopoletin, IAA (of host and pathogen origin), ethylene, soluble protein, aromatic amino acids, chlorogenic acid, polymerized polyphenols, and a compound similar to the so-called inhibitor-β. The activities of the following enzymes or enzyme systems are increased: polyphenoloxidase, peroxidase, catalase, and possibly phenylalanine ammonia lyase.

The changes in enzyme systems, respiration patterns, and metabolites in plants affected by *P. solanacearum* are similar in many respects to the changes caused by other bacterial plant pathogens. With the exception of the extracellular polysaccharide and effects of cell wall degrading enzymes, symptoms of the disease are mainly attributable to response of the host. The chemical nature of those pathogen metabolites that switch on the enzymatic machinery that results in a susceptible or resistant reaction have not been determined for *P. solanacearum* or any of the other bacterial pathogens. The inducer for the hypersensitive

reaction and for the increased resistance resulting from injection of dead cells is also unknown. The characterization of the specific inducers formed in the earliest stages of disease development is essential in expanding our knowledge of the disease process. It is obvious that the very first interactions of the host and the pathogen need to be examined much more intensively than they have been studied in the past.

There are few instances of a demonstrated correlation between ability of a pathogen to form a specific injurious substance and level of virulence. The relationship between slime formation and virulence in *P. solanacearum* is analogous to that between capsule formation and virulence in certain animal and human pathogens such as *Pneumococcus*. The capsular polysaccharide of animal and human pathogens protects the invading bacterium against the action of phagocytes, antibodies, and the reticulo-endothelial system of the mammal. The polysaccharide of the virulent wilt bacterium, in addition to its role in wilt induction may function in a manner similar to that of capsular material for animal pathogens in its relation to the cell and in the effects of its inherent chemical components. The specific functional protective value of a slime layer for a plant pathogen is presumably quite different from that of an animal pathogen, however.

With the new techniques available that permit identification and characterization of minute quantities of plant and pathogen products there is hope of unravelling the extremely complex changes that occur in a diseased plant. The necessity of separating these products and of detecting inducing and repressing substances at extremely low levels will require the greatest precision and skill. In the hands of plant pathologists without the depth of training essential for biochemical studies, there is danger that the tools will exceed the capacity of the users to interpret the data. It is obvious that increased involvement of biochemists, plant physiologists, geneticists and molecular biologists in the examination of disease processes is essential. Resolution of the unique biological system represented by two interacting organisms requires a higher level of precision and analytic ability than the study of the organisms as separate entities. Bacterial-plant systems provide many unique advantages in elucidating these relationships. From such studies can come greater insight into normal growth and development of plants.

March 17, 1969

REFERENCES CITED

Albersheim, P., D. J. Nevins, and P. D. English. 1968. The biochemistry of plant cell wall polysaccharides: a basis for disease resistance. 297-312. *In* "Biochemical Regulation in Diseased Plants or Injury." T. Hirai, Z. Hidaka, and I. Uritani (Eds.). Phytopathological Soc. Japan, Tokyo. 351 p.

Allen, P. J. 1966. The role of the host in the development of disease symptoms—A Review. Phytopathology 56:255-260.

Averre, C. W., III, and A. Kelman. 1964. Severity of bacterial wilt as influenced by the ratio of virulent to avirulent cells of *Pseudomonas solanacearum* in inoculum. Phytopathology 54:779-783.

Bateman, D. F. 1968. The enzymatic maceration of plant tissue. Neth. J. Plant Pathol. 74:(Suppl.) 67-80.

Bateman, D. F., and R. L. Millar. 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. 4:119-146.

Bawden, F. C. 1964. Plant Viruses and Virus Diseases. Fourth Ed. The Ronald Press, New York. 361 p.

Beckman, C. A. 1964. Host response to vascular infection. Annu. Rev. Phytopathol. 2:231-252.

Beckman, C. H., W. A. Brun, and I. W. Buddenhagen. 1962. Water relations in banana plants infected with *Pseudomonas solanacearum*. Phytopathology 52: 1144-1148.

Bollard, E. G., and R. E. F. Matthews. 1966. The physiology of infectious disease. *In* Plant Physiology IV B:417-450. Steward, F. C. (Ed.). Academic Press, New York. 599 p.

Braun, A. C. 1955. A study on the mode of action of the wildfire toxin. Phytopathology 45:659-664.

Braun, A. C. 1962. Tumor inception and development in the crown gall disease. Annu. Rev. Pl. Physiol. 13:533-558.

Buddenhagen, I., and A. Kelman. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Ann. Rev. Phytopathol. 2:203-230.

Burg, S. P. 1962. The physiology of ethylene formation. Annu. Rev. Pl. Physiol. 13:265-302.

Dean, Margaret and R. K. S. Wood. 1968. Maceration by *Erwinia aroideae*. Neth. J. Pl. Pathol. 74 (Suppl. 1):81-85.

DeVay, J. E., F. L. Lukezic, S. L. Sinden, H. English, and D. L. Coplin. 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. Phytopathology 58:95-101.

Diener, T. O. 1963. Physiology of virus infected plants. Annu. Rev. Phytopathol. 1:197-218.

Dimond, A. E. 1955. Pathogenesis in the wilt diseases. Annu. Rev. Pl. Physiol. 6:329-350.

Dimond, A. E. 1967. Physiology of wilt disease. 100-120. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul, Minn. 372 p.

Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. Phytopathology 43:229-235.

Dowson, W. J. 1957. Plant Diseases Due to Bacteria. Second Ed. Cambridge Univ. Press, Cambridge. 231 p.

Ercolani, G. L. 1968. Schizomycetes. Vol. III. Ed. G. Goidanich Manuale di Patalogia Vegetable. Edizioni Agricole, Bologna. 456 p.

Farkas, G. L., and Z. Kiraly. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopathol. Z. 44:105-150.

Freebairn, H. T., and I. W. Buddenhagen. 1964. Ethylene production by *Pseudomonas solanacearum*. Nature 202:313-314.

Gäumann, E. 1951. Some problems of pathological wilting in plants. Advan. Enzymol. 11:401-435.

Gehring, F. 1962. Untersuchungen über den Infektionsverlauf einer durch *Pectobacterium parthenii* (Starr) Hellmers var *dianthicola* Hellmers verursachten Nelkenbakteriose. Phytopathol. Z. 43:383-407.

Goodman, R. N. 1968. The hypersensitive reaction in tobacco: a reflection of changes in host cell permeability. Phytopathology 58:872-873.

Goodman, R. N., Z. Kiraly, and M. Zaitlin. 1967. The biochemistry and physiology of infectious plant disease. D. Van Nostrand Co., Princeton. 354 p.

Goto, M., and N. Okabe. 1959. Studies on the cellulolytic enzymes of phytopatho-

genic bacteria. Parts 1, 2, 3. Ann. Phytopathol. Soc. Japan 24:182-193 (1959); 25:27-31 (1960).

Grieve, B. J. 1941a. Studies in the physiology of host-parasite relations. I. The effect of *Bacterium solanacearum* on water relations of plants. Proc. Roy. Soc. Victoria 53:268-299.

Grieve, B. J. 1941b. Epinastic response induced in plants by *Bacterium solanacearum* E.F.S. Ann. Botan. (London) N.S. 3:587-600.

Harris, H. A. 1940. Comparative wilt induction by *Erwinia tracheiphila* and *Pseudomonas stewarti*. Phytopathology 30:625-638.

Helgeson, J. P. 1968. The cytokinins. Science 161:974-981.

Hildebrand, D. C., and M. N. Schroth. 1964. Arbutin-hydroquinone complex in pear as a factor in fire blight development. Phytopathology 54:640-645.

Horsfall, J. G., and A. E. Dimond (Eds.). 1959. The diseased plant. *In* "Plant Pathology." Vol. 1. Academic Press, New York. 674 p.

Horsfall, J. G., and A. E. Dimond (Eds.). 1960. The pathogen. *In* "Plant Pathology." Vol. 2. Academic Press, New York. 715 p.

Husain, A., and A. Kelman. 1958. Relation of slime production to mechanism of wilting and pathogenicity of *Pseudomonas solanacearum*. Phytopathology 48: 155-165.

Husain, A., and A. Kelman. 1959. Tissue is disintegrated. pp. 144-182. *In* "Plant Pathology." Vol. 1. J. G. Horsfall and A. E. Dimond (Eds.). Academic Press, New York. 674 p.

Keen, N. T., P. H. Williams, and C. D. Upper. 1969. A reevaluation of the protease from *Pseudomonas lachrymans:* isolation of a fraction producing noncatalytic solubilization of proteins in trichloroacetic acid. Phytopathology 59:703-704.

Keen, N. T., P. H. Williams, and J. C. Walker. 1967a. Protease of *Pseudomonas lachrymans* in relation to cucumber angular leaf spot. Phytopathology 57: 263-271.

Keen, N. T., P. H. Williams, and J. C. Walker. 1967b. Characterization of a protease produced by *Pseudomonas lachrymans*. Phytopathology 57:257-262.

Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. North Carolina Agr. Expt. Sta. Tech. Bull. 99. 194 p.

Kelman, A. 1954. The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. Phytopathology 44:693-695.

Kelman, A., and E. B. Cowling. 1965. Cellulolytic enzymes of *Pseudomonas solanacearum* in relation to pathogenesis. Phytopathology 55:148-155.

Klement, Z., and R. N. Goodman. 1967. The hypersensitive reaction to infection by bacterial plant pathogens. Annu. Rev. Phytopathol. 5:17-44.

Kosuge, T., and T. T. Kuo. 1968. Metabolism of indole-3-acetic acid by the phytopathogen, *Pseudomonas savastanoi*. pp. 313-319. *In* "Biochemical Regulation in Diseased Plants or Injury." T. Hirai, Z. Hidaka, and I. Uritani (Eds.). Phytopathological Soc. Japan, Tokyo. 351 p.

Kuc, J. 1967. Shifts in oxidative metabolism during pathogenesis. 183-202. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul. 372 p.

Leach, J. G., V. G. Lilly, H. A. Wilson, and M. R. Purvis, Jr. 1957. Bacterial polysaccharides: the nature and function of the exudate produced by *Xanthomonas phaseoli*. Phytopathology 47:113-120.

Lippincott, B., and J. A. Lippincott. 1969. Bacterial attachment to a specific wound site as an essential stage in tumor initiation by *Agrobacterium tumefaciens*. J. Bacteriol. 97:620-628.

Lovrekovich, L., H. Lovrekovich, and M. A. Stahmann. 1967. Inhibition of phenol oxidation by *Erwinia carotovora* in potato tuber tissue and its significance in disease resistance. Phytopathology 57:737-742.

Lovrekovich, L., H. Lovrekovich, and M. A. Stahmann. 1968. The importance of peroxidase in the wildfire disease. Phytopathology 58:193-198.

Lozano, J. C., and L. Sequeira. 1968. Differentiation of races of *Pseudomonas*

solanacearum by a hypersensitive reaction of tobacco leaves. (Abstr.). Phytopathology 58:1058.

Ludwig, R. A. 1960. Toxins. *In* "Plant Pathology." Vol. 2. The Pathogen. J. G. Horsfall and A. E. Dimond (Eds.). Academic Press, New York.

Maine, E. C. 1960. Physiological responses in the tobacco plant to pathogenesis by *Pseudomonas solanacearum*, causal agent of bacterial wilt. Doctoral Thesis. N. C. State Univ., Raleigh, N. C.

McNew, G. L. 1960. The nature, origin and evolution of parasitism. pp. 19-69. *In* "Plant Pathology." Vol. 2. J. G. Horsfall and A. E. Dimond (Eds.). Academic Press, New York. 713 p.

Owens, L. D., S. Guggenheim, and J. L. Hilton. 1968. Rhizobium synthesized phytotoxin: an inhibitor of β-cystathionase in *Salmonella typhimurium*. Biochim. Biophys. Acta 158:219-225.

Patel, P. N., and J. C. Walker. 1963. Changes in free amino acid and amide content of resistant and susceptible beans after infection with the halo blight organism. Phytopathology 53:522-528.

Pegg, G. F., and L. Sequeira. 1968. Stimulation of aromatic biosynthesis in tobacco plants infected by *Pseudomonas solanacearum*. Phytopathology 58:476-482.

Phelps, R. H., and L. Sequeira. 1967. Synthesis of indoleacetic acid by cell free systems from virulent and avirulent strains of *Pseudomonas solanacearum*. Phytopathology 57:1182-1190.

Pringle, R. B., and R. P. Scheffer. 1964. Host specific plant toxins. Annu. Rev. Phytopathol. 2:133-156.

Rai, P. V., and G. A. Strobel. 1969. Phytotoxic glycopeptides produced by *Corynebacterium michiganense*. II. Biological properties. Phytopathology 59:53-57.

Rubin, B. A., and Y. V. Artsikovskaya. 1963. Biochemistry and physiology of plant immunity. English translation by Helen Wareing. Macmillan Co., N.Y. 358 p.

Rudolph, K., and M. A. Stahmann. 1964. Some investigations on the proteins of bean leaves infected by *Pseudomonas phaseolicola*. Z. Pflanzenkrankh. 71: 107-112.

Scheffer, R. P., and R. B. Pringle. 1967. Pathogen produced determinants of disease and their effects on host plants. pp. 217-236. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul. 372 p.

Sequeira, L. 1963. Growth regulators in plant disease. Annu. Rev. Phytopath. 1:5-30.

Sequeira, L. 1964. Inhibition of indoleacetic acid oxidase in tobacco plants infected by *Pseudomonas solanacearum*. Phytopathology 54:1078-1083.

Sequeira, L. 1965. Origin of indoleacetic acid in tobacco plants infected by *Pseudomonas solanacearum*. Phytopathology 55:1232-1236.

Sequeira, L. 1969. Synthesis of scopolin and scopoletin in tobacco plants infected by *Pseudomonas solanacearum*. Phytopathology 59:473-478.

Sequeira, L., and A. Kelman. 1962. Accumulation of growth substances in plants infected by *Pseudomonas solanacearum*. Phytopathology 52:439-448.

Sinden, S. L., and R. D. Durbin. 1968. Glutamine synthetase inhibition: possible mode of action of wildfire toxin from *Pseudomonas tabaci*. Nature 219:379-380.

Sinden, S. L., and R. D. Durbin. 1969. Some comparisons of chlorosis-inducing Pseudomonad toxins. Phytopathology 59:249-250.

Spencer, J. F. T., and P. A. Gorin. 1961. The occurrence in the host plant of physiologically active gums produced by *Corynebacterium insidiosum* and *Corynebacterium sepedonicum*. Can. J. Microbiol. 7:185-188.

Stahmann, M. A. 1967. Influence of host-parasite interactions on proteins, enzymes, and resistance. 357-372. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul. 372 p.

Stapp, C. 1961. Bacterial Plant Pathogens. Oxford Univ. Press, London. 292 p.

Stapp, C. 1965. Die bakterielle Schleimfäule und ihr Erreger *Pseudomonas solanacearum*. Zentralbl. f. Bakt., Parasiten. Infektionskr. u. Hygiene 119:166-190.

Starr, M. P. 1959. Bacteria as plant pathogens. Annu. Rev. Microbiol. 13:211-238.
Steadman, J. R., and L. Sequeira. 1969. A growth inhibitor from tobacco and its possible involvement in pathogenesis. Phytopathology 59:499-503.
Strobel, G. A., and W. M. Hess. 1968. Biological activity of a phytotoxic glycopeptide produced by *Corynebacterium sepedonicum*. Plant Physiology 43:1673-1688.
Sutton, J. C., and P. H. Williams. 1968. Xylem plugging and black rot lesion development in cabbage. (Abstr.) Phytopathology 58:1069.
Tani, T. 1967. The relation of soft rot caused by pathogenic fungi to pectic enzyme production by the host. pp. 40-58. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul. 372 p.
Thimann, K. V., and T. Sachs. 1966. The role of cytokinins in the "fasciation" disease caused by *Corynebacterium fascians*. Amer. J. Bot. 53:731-739.
Tseng, T.-C., and D. F. Bateman. 1968. Production of phosphatidases by phytopathogens. Phytopathology 58:1437-1438.
Uritani, I., T. Asahi, T. Minamikawa, H. Hyodo, K. Oshima, and M. Kojima. 1967. The relation of metabolic changes in infected plants to changes in enzymatic activity. 342-356. *In* "The Dynamic Role of Molecular Constituents in Plant-Parasite Interaction." C. J. Mirocha and I. Uritani (Eds.). Bruce Publ. Co., St. Paul. 372 p.
U. S. Dept. Agr. 1960. Index of Plant Diseases in the United States. U.S.D.A. Agriculture Handbook No. 165. 531 p.
Wheeler, H., and Penelope Hanchey. 1968. Permeability phenomena in plant disease. Annu. Rev. Phytopathology 6:331-350.
Wheeler, H., and H. H. Luke. 1963. Microbial toxins and plant disease. Annu. Rev. Microbiol. 17:223-242.
Wood, R. K. S. 1960. Pectic and cellulolytic enzymes in plant disease. Annu. Rev. Pl. Physiol. 11:299-322.
Wood, R. K. S. 1967. Physiological Plant Pathology. Blackwell Scientific Publications, Oxford. 570 p.
Yarwood, C. E. 1967. Response to parasites. Annu. Rev. Pl. Physiol. 18:419-438.

How Insects Damage Crops

Perry L. Adkisson

The topic "How Insects Damage Crops" appears deceptively simple. One could say in the simplest, most concise terms, and with certainty, that insects do direct damage to crops by feeding on or within individual plants of a crop. However, the man we honor, Professor Samuel W. Johnson, surely would have preferred to examine this problem in more detail. He would have been interested in the impact of the war between men and insects on the total agro-ecosystem. Some of the side-effects of pest control in the final analysis are more important than the primary effect of controlling a specific pest by the most direct means.

The agro-ecosystem has been defined by Smith and Reynolds (1969) as

> . . . a unit composed of the total complex of organisms in a crop-producing area together with the over-all conditioning environment and as further modified by the various agricultural, industrial, recreational and social activities of man.

Smith and Reynolds (1969) also state:

> In the practical analysis of an agro-ecosystem for pest management, emphasis is placed on the populations of the pest species, their competitors, the organisms that prey on them, their main and alternative food supplies, and the manner in which other elements of the environment modify all of these. The determination of insect pest population levels is broadly under the influence of the agro-ecosystem, and a knowledge of how this influence operates is essential to an ecological approach to pest control.

The Cotton Agro-Ecosystem

The present nature of the insect pest complex of cotton (*Gossypium*) in the Lower Rio Grande Valley of Texas provides a vivid illustration of how man's manipulation of the agro-ecosystem may greatly complicate problems of pest management. Although this example will deal with insect control, the fundamental problems involved could apply to any pest control scheme in any agro-ecosystem.

The problem in the Lower Rio Grande Valley revolves around insect control under conditions where cotton is the principal crop. Here the

agro-ecosystem is made up of the population of cotton plants, the soil and its organisms, the chemical and physical environment, an energy input from the sun, and other inputs due to man. It typically includes a group of agricultural fields, marginal areas, woods, streams, and uncultivated land. Some of the cultivated fields have crops other than cotton such as grain sorghum, citrus, vegetables or pasture.

Cotton, the principal source of income to the area, is attacked by many insects, including the boll weevil (*Anthonomus grandis* Boheman), bollworm (*Heliothis zea* Boddie), pink bollworm (*Pectinophora gossypiella* Saunders), tobacco budworm (*Heliothis virescens* Fabricus), aphids (*Aphis gossypii* Glover), and various species of spider mites.

Prior to World War II, the boll weevil was the primary pest of cotton in the area. Cotton was mainly grown without fertilization or irrigation. The boll weevil was controlled with calcium arsenate dust and yields were relatively low. The bollworm occurred sporadically and was considered a secondary pest of cotton. Literature of those days almost never mentioned the tobacco budworm on cotton, although it was known to be a minor pest.

Impact of Chlorinated Hydrocarbons

Shortly after World War II, the new synthetic chlorinated hydrocarbon insecticides were introduced into the area and had a spectacular effect on cotton production. These insecticides possessed two qualities of great importance: (1) high initial toxicity to insect pests, and (2) sufficient persistence to control newly emerging insects or insects migrating from untreated into treated areas (Knipling, 1967).

For the first time, cotton producers were able to effectively control the boll weevil and entomologists also were able to make the first accurate appraisal of the value of effective insect control to cotton production. The impact of DDT, benzene hexachloride (BHC), toxaphene, dieldrin, endrin, and other new insecticides was to stimulate an unprecedented demand by producers for almost complete control of the boll weevil and all other damaging insect pests of cotton. It was now profitable for them to use fertilizers, irrigation and other practices. Spectacular increases in yields were obtained and maintained at high profit levels for many years.

On the surface, insect control in the Valley during this period seemed to be without major problems. Toxaphene, BHC, dieldrin, and endrin provided very effective control of the boll weevil, but these materials also killed a broad spectrum of insects including the major predators and parasites of the bollworm and tobacco budworm. It was quickly recognized that these lepidopterous pests could be of major economic importance when released from natural control. The solution to this problem appeared to be relatively simple. DDT, though not effective against the boll weevil, was highly toxic to the bollworm and tobacco budworm. Therefore, DDT was added to the boll weevil insecticides as

a preventive measure. Mixtures of insecticides such as toxaphene and DDT provided such effective control that producers were not troubled with outbreaks of the bollworm and tobacco budworm. In fact, they did not recognize them as serious pests. The boll weevil was the primary pest, the control of which necessitated the intensive use of insecticides.

Impact of Organophosphorus Insecticides

The Lower Rio Grande Valley was intensively dusted and sprayed with chlorinated hydrocarbon insecticides for approximately 15 years. No serious problems in control were experienced until the late 1950's, at which time the boll weevil became resistant to the chlorinated hydrocarbon insecticides. This problem was resolved by a switch to the organophosphorus insecticides, mainly methyl parathion. However, when applied at dosages sufficient to control the boll weevil these insecticides were ineffective against the bollworm and tobacco budworm; thus, DDT, again was added for the control of these two pests. Excellent control of all three pests was obtained once again. However, this situation did not last long. In 1960 difficulties were experienced in controlling the bollworm and tobacco budworm. By 1962 these pests could not be controlled with DDT or other chlorinated hydrocarbon or carbamate insecticides. Data, summarized in Table 1, for the tobacco budworm provide an example showing the rapidity of the development of resistance to insecticides by these pests (Adkisson, 1964, 1967, 1968; Adkisson and Nemec 1965, 1966). The total pest control situation suddenly reversed. Now the bollworm and the tobacco budworm became primary pests while the boll weevil faded to secondary importance.

The problem of bollworm and tobacco budworm resistance to DDT and related compounds was resolved by increasing dosages of methyl parathion from 0.50 lb/acre which had been used for weevil control to 1.0 to 2.0 lb. per acre. Of course, DDT was not needed for cotton insect pest control. The increased dosage of methyl parathion provided producers with an effective method for controlling the bollworm-budworm

Table 1. Development of resistance by the tobacco budworm to certain chlorinated hydrocarbon and carbamate type insecticides during the period 1961 to 1965

Compound	LD_{50}, Mg/G				
	1961	1962	1963	1964	1965
DDT (Resist.)	16.12	36.72	16.51	16.51	
DDT (Susc.)	0.13				
Endrin	0.06	0.12	0.20	1774.85	12.94
Carbaryl	0.30	0.60	15.64	12.53	54.47
Strobane® (terpene polychlorinates)-DDT	0.73	0.77	0.51	1.72	11.12
Toxaphene-DDT	0.47	0.63	0.57	2.97	3.52

complex. This dosage proved extremely deadly to the boll weevil and the use of methyl parathion at these dosages has practically eradicated the pest from the Valley. At present, producers in the area do not think of the boll weevil as a major pest, although it persists each year in low numbers. An immediate effect of increasing dosage rates of insecticides was to increase the cost of production. Yields remained high but profits decreased. This situation prevailed until 1968. Control now was directed mainly at the bollworm and tobacco budworm, two pests formerly of secondary importance, while the boll weevil had faded to insignificance.

In 1968, the situation began to change drastically. The tobacco budworm population in the Valley showed the first evidence of developing resistance to the organophosphorus insecticides. Many producers treated fields 15 to 18 times with methyl parathion and even then suffered great losses in yields. Others produced relatively high yields but saw their already small profit margin shrink even more.

Tests conducted in the laboratory at Texas A&M University confirmed field observations that the tobacco budworm populations in the Valley had developed resistance to methyl parathion. This resistance, combined with great populations of budworms, made control extremely difficult. As shown in Figure 1, dosages of methyl parathion which had killed all of a test population of budworms in 1967 killed less than 50% of those treated in 1968 (Nemec and Adkisson, 1969). Furthermore, control leveled off between the 80 and 90% mortality level. That is, a large increase in dosage did not appreciably increase the percentage of insects killed. These data indicated that approximately 15% of the budworm population had become extremely resistant to the organophosphorus insecticides.

Perhaps, the impact of this development can best be shown by a simplified model of insect population dynamics. Insect populations seldom are stable; they are dynamic, either increasing or decreasing. If the birth-rate exceeds the death-rate the size of the population increases and if the reverse occurs (if the death-rate exceeds the birth-rate) the population decreases. Table 2 shows the level of control that is demanded if a population is increasing at a 5- or 10-fold rate per generation. At a 5-fold rate of increase per generation, insecticide treatments which kill only 80% of the population would in effect do no more than maintain the *status quo*. The population would neither decrease nor increase. In fact, 90% control must be obtained to reduce the population by 50%. At a 10-fold rate of increase, 90% control would only be sufficient to keep the population from increasing.

The population model in Table 2 provides an excellent explanation for part of the problem in 1968. Tobacco budworm populations as great as 70,000 larvae per acre were encountered in the Lower Rio Grande Valley in 1968. Approximately 2,500 larvae per acre do economic damage (Adkisson et al., 1964). Thus, 90% control of a population of 70,000 per acre would not be effective in preventing losses in yield.

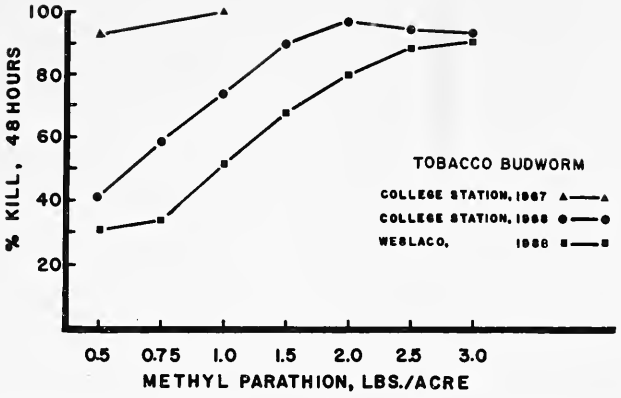


Fig. 1. Effects of graded dosages of sprays of methyl parathion against tobacco budworm larvae from Weslaco and College Station, Texas caged on treated plants, 1968.

The problem now has come full circle. A secondary pest has achieved more importance than a primary pest, and the arsenal of effective insecticides has almost been exhausted. If the organophosphorus insecticides fail completely, there presently do not appear to be any new materials being developed to take their place.

Crisis Phase

The present situation in the Lower Rio Grande Valley of Texas meets all the criteria of the crisis phase in cotton pest control. This has been defined by Smith (1969) as follows:

> After a variable number of years in the exploitation phase of cotton production and heavy use of pesticides, a series of events occur. More frequent applications of pesticides are needed to get effective control. The treatments start earlier in the cotton growing season and extend later into

Table 2. Effects of two levels of control on an insect population that is increasing at a 5- or 10-fold rate per generation

Number of Larvae/Acre Prior to Treatment	Per Cent Control	Assumed Rate of Increase per Generation	Number of Larvae/Acre after Treatment	Per Cent Reduction
10,000	80	5	10,000	0
10,000	90	5	5,000	50
10,000	90	10	10,000	0

> the harvest period. It is noted that the pest populations now resurge rapidly after treatment to new higher levels. The pest populations gradually become so tolerant of the pesticide that it is useless. Another insecticide is substituted and the pest populations become tolerant to it too, but this happens more rapidly than with the first chemicals. At the same time, pests that never caused damage in the area previously, or only occasionally, become serious and regular ravagers of the cotton fields. This combination of pesticide resistance, pest resurgence, and unleashed secondary pests causes greatly increased production costs.

Smith further defines the disaster-phase,

> which occurs when pesticide usage increases production costs to the point where cotton can no longer be grown profitably. At first, marginal land and marginal farmers are removed from production. Eventually, cotton is no longer profitable to produce in the area.

The data reported in Table 3 provide a graphic illustration that cotton production in the Lower Rio Grande Valley of Texas is in the crisis phase and nearing the disaster phase. These data show the relative levels of resistance to the organophosphorus insecticides that have developed in various populations of tobacco budworms (Nemec and Adkisson, 1969). The tobacco budworm has developed a 4-fold level of resistance to methyl parathion in Central Texas, and a 5- to 9-fold level at Weslaco and Brownsville in the Lower Rio Grande Valley of Texas. More importantly, tobacco budworm populations near Tampico, Mexico show a 31-fold level of resistance to methyl parathion when compared to the 1964 College Station population. This resistance occurs in an area just 200 miles south of the Texas border.

Certain areas of Mexico apparently have reached the disaster stage. Cotton production in the Matamoros-Reynosa area bordering the Lower Rio Grande Valley of Texas declined from 710,715 acres in 1960 to 24,178 acres by 1967 (Table 4). Most of this acreage was moved to new

Table 3. Comparative resistance of tobacco budworms collected from various locations to methyl parathion

Location	Year	LD_{50}, MG/G Larva	Increase in resistance over 1964, Col. Sta.
College Station	1964	0.01	
College Station	1968	0.04	4X
Weslaco	1968	0.05	5X
Brownsville[a]	1968	0.09	9X
Tampico[a]	1968	0.31	31X

[a] Unpublished data by D. A. Wolfenbarger, USDA, ENT, Brownsville, Texas.

Table 4. Reductions in cotton acreage in certain areas of Mexico following the development of resistance by the bollworm and tobacco budworm to certain insecticides[1]

Year	Acres	Bales
	Matamoros–Reynosa	
1960	710,715	370,382
1961	613,640	240,609
1962	499,790	362,197
1963	509,058	159,515
1964	191,780	54,066
1965	102,605	50,207
1966	43,515	18,183
1967	24,178	15,957
	Tampico[2]	
1966	500,000	?
1968	200,000	?
1969	100,000	10,000

[1] Data provided by Dr. M. J. Lukefahr, Head, USDA Entomology Research Laboratory, Brownsville, Texas.

[2] Acreage was moved from Matamoros to new land in Tampico during mid-1960's.

lands near Tampico. In 1966 there were approximately 500,000 acres planted to cotton in the Tampico area. This declined to 200,000 acres in 1968. Present indications are that this acreage will decline to less than 100,000 acres in 1969. This has occurred mainly because the greatly increased usage of insecticides in attempts to control resistant bollworms and budworms plus substantial yield losses caused by these pests has made it unprofitable to produce cotton in these areas of Mexico.

A secondary effect of the crisis and disaster phase which looms as major in importance is the environmental hazard caused by the increased use of greater doses of more toxic insecticides. Growers in the Lower Rio Grande Valley have decreased treatment intervals in many instances from 5 to 3 or 4 days and have increased doses of methyl parathion or mixtures of methyl and ethyl parathion to 2 and some cases 3 lbs/acre per application. As a consequence of this action, there has been a great increase in the number of cases of pesticide poisoning reported in the area. Reich *et al.* (1968) reported that 275 cases of pesticide poisoning occurred in the Lower Rio Grande Valley of Texas between 1960 and 1966. The majority of the cases reported involved poisoning by the organophosphorus insecticides. A study of 129 well-documented cases of acute pesticidal intoxication showed that the majority of the poisonings occurred during the summer months and principally involved aerial pesticide applicators and farm workers.

Fortunately, there have been no deaths directly attributed to the agricultural application of organophosphorus insecticides. However, the

problems of safety and environmental contaminations are great. Many non-farm residents are complaining about heavy use of insecticides in fields adjacent to the small towns in the Valley. Also, insecticidal drift from cotton into citrus orchards has caused major upsets in the biological control of certain arthropod pests of this crop.

Implications

Many things may be learned from the present insect problem in Texas. The first is that there are few "natural" ecosystems left in which natural control agents will hold pest populations in balance. In the Lower Rio Grande Valley of Texas the first ecological disturbance was the plowing up of the land and the establishment of vast acreages of crops, especially cotton. The second major disruption was the invasion of cotton by the boll weevil in 1892. The third disruption was produced by the insecticides used to control the boll weevil. This allowed secondary pests to become of primary importance through the destruction of beneficial insects and the development of insecticide resistant pest populations. Attempts to deal with this problem have led to a fourth disturbance—the increased pollution of the environment with pesticides. This pollution not only affects the composition of the insect populations in cotton and citrus orchards but is becoming increasingly serious for the human population in the area.

The lessons being learned in entomology may well serve as examples for what may happen in pest control schemes which depend almost entirely on chemicals. For example, problems similar to those occurring in insect control appear to be surfacing in the herbicidal control of weeds. A primary weed pest is controlled with a herbicide. This pest then is succeeded by a secondary weed species which soon becomes primary in importance. A mixture of chemicals is used for these and then third and fourth order weeds soon become more important than the first two (Frans, 1969). This appears to closely duplicate the events experienced in the control of the insect pests of cotton. The development of resistance to herbicides by certain weed species must not be far over the horizon and the contamination of soil and water by herbicides is an established fact.

Future Policies

So much for the problems. Are there solutions? I think there are. Entomologists will have to develop integrated control measures for insects which will lessen dependence on insecticides. Emphasis will be placed on the selective uses of insecticides both in dose and choice of insecticide. This should minimize disruptions caused by destruction of beneficial arthropods. There will soon be suitable cotton varieties that possess some resistance or tolerance to certain key species of minor economic insects. Producers will go back to proven cultural practices that reduce overwintering and early spring emerging insects. They

may have to settle for less than the maximum yield in order to produce a profitable crop. But, they will produce a profit.

I am confident that we can deal with pest control problems if we are not unduly hindered by persons concerned only with environmental pollution. We must be aware of the pressures that will be brought to bear on us as agricultural researchers. Producers will demand maximum yield and anti-pollutionists will demand minimum usage of pesticides. In fact, some will demand that their use be banned altogether. We, as agricultural researchers, should impress upon the producer, the general public and the legislatures that we are the scientists who must solve this problem. This is necessary if the United States is to have a strong farm economy and an adequate supply of low priced wholesome food. If there is a balance to be struck in this situation, the agricultural researchers of the land-grant colleges and the U. S. Department of Agriculture are the ones to show the way. To do this, we must take the leadership and show by example that the responsibility for the proper conduct of pest management is and should be ours. This is demanded if crop production is to be maintained and contamination of the environment by pesticides is to be held within safe limits.

Interdisciplinary teams of ecologists, crop protection specialists, breeders, conservationists, and economists must be organized within the land-grant colleges and agricultural experiment stations to study in depth the total ecology of the various ecosystems these institutions serve. If we agricultural researchers are not provided the means to do this, I believe we will see our various professions and even our universities themselves come under attack from various non-agricultural groups. This could have a devastating result on the agricultural production and economy of this country. Let us not apologize for the past but begin the research needed for the future.

April 21, 1969

LITERATURE CITED

Adkisson, P. L. 1964. Comparative effectiveness of several insecticides for controlling bollworms and tobacco budworms. Texas Agr. Exp. Sta. Misc. Publ. 709. 13 p.

———— 1967. The development of resistance by the tobacco budworm to mixtures of toxaphene or Strobane plus DDT. J. Econ. Entomol. 60: 789-91.

———— 1968. Development of resistance by the tobacco budworm to endrin and carbaryl. J. Econ. Entomol. 61: 37-40.

Adkisson, P. L. and S. J. Nemec 1965. Efficiency of certain insecticides for killing bollworms and tobacco budworms. Texas Agr. Exp. Sta. Prog. Rpt. 2357. 11 p.

———— 1966. Comparative effectiveness of certain insecticides for killing bollworms and tobacco budworms. Texas Agr. Exp. Sta. Bull. 1048. 4 p.

Adkisson, P. L., R. L. Hanna and C. F. Bailey 1964. Estimates of the actual numbers of *Heliothis* larvae per acre in cotton and their relation to the fruiting cycle and yield of the host. J. Econ. Entomol. 57: 657-63.

Frans, R. E. 1969. Changing ecology of weeds in cotton fields. Proc. 1969 Beltwide Cotton Prod.—Mech. Conf. (New Orleans, La.) Natl. Cot. Council Amer. pp. 29-31.

Knipling, E. F. 1967. The important role of residual insecticides in food production in the U.S.A. Symposium on Science and Technology of Residual Insecticides in Food Production with Special Reference to Aldrin and Dieldrin (Shell Chemical Co., St. Charles, Ill., Nov. 2-3, 1967). pp. 41-50.

Nemec, S. J. and P. L. Adkisson 1969. Laboratory tests of insecticides for bollworm, tobacco budworm, and boll weevil control. Texas Agr. Exp. Sta. Prog. Rept. 2674.5 p.

Reich, G. A., G. L. Gallaher, and J. S. Wiseman 1968. Characteristics of pesticide poisoning in South Texas. Texas Medicine 64: 56-58.

Smith, R. F. 1969. Patterns of crop protection in cotton ecosystems. Lecture presented to Cotton Symposium on Insect and Mite Control Problems and Research in California, Berkeley, Calif., March 12-13, 1969. p. 6.

——— 1969. Effect of manipulation of cotton agro-ecosystems on insect pest populations. Ecology and International Development, M. T. Farvar and J. P. Milton (ed.). Natural History Press. (In press.)

How Science Grows

Leland J. Haworth

The theme of the Centennial Lectures is growth, reproduction, development, and constraints on growth. My theme will be much the same except that the subject will be growth of science rather than crops. The importance of this question was aptly put by Derek Price who noted that:

> It must be recognized that the growth of science is something very much more active, much vaster in its problems, than any other sort of growth happening in the world today. For one thing, it has been going on for a longer time and more steadily than most other things. More important, it is growing much more rapidly than anything else. All other things in population, economics, and nonscientific culture, are growing so as to double in roughly every human generation or say thirty to fifty years. Science in America is growing so as to double in only ten years.

Because there seem to be as many ideas of what science is as there are spokesmen for science, we ought to define our terms before we talk about the growth of science. A somewhat earthy definition which dates from the 18th century, but which rings true today was made by the poet, Goethe, who stated:

> To one man it is the highest thing, a heavenly goddess; to another it is a productive and proficient cow, who supplies him with butter.

For the purposes of this lecture, we need a less poetic explanation, and I will use as a working definition of science: "that organized knowledge and understanding of the general laws of nature which has been accumulated by the collection of data through observation and experiment and the formulation and testing of hypotheses."

Although science seems to many to have suddenly matured and thrust itself into the central stage of human experience, in actuality its progress has been gradual, steady, and incremental. Occasionally great flashes of insight and intuition flood the scientific landscape with the light of scientific discovery, but we must not lose sight of the fundamental trends that develop gradually, over a long period of time. I have selected for discussion, three distinguishable, though closely related trends which I believe are fundamental, with important consequences for the short- and long-term future growth of science.

These trends in the evolutionary growth of science may be described as:

1) The increasing association of scientists within institutions of various types and purposes.

2) The present trend toward unity in science following a long period of divergence into largely disassociated disciplines.

3) The growing interrelationships between science and technology.

Science and the Individual

Perhaps our examination of these three trends in the growth of science can best begin by viewing the changes from individual investigations to groupings of individuals in institutions for the study of science. That science was and is highly individualistic has been commented on by many eloquent observers. For example, Warren Weaver noted that:

> Science is not technology, it is not some mysterious cult, it is not a great mechanical monster. Science is an adventure of the human spirit. It is essentially an artistic enterprise, stimulated largely by curiosity, served largely by disciplined imagination, and based largely on faith in the reasonableness, order, and beauty of the universe of which man is part.

Because science is "an adventure of the human spirit," it naturally follows, in the words of Max Born that:

> Faith, imagination, intuition are decisive factors in the progress of science.

Clearly, these characteristics are highly individualistic, but one might reasonably ask why this is so accentuated in science. The answer to this question, if there is one, may be found in the use of the scientific method. At one time it was postulated that science was merely a collection of observational facts linked together by induction. We now know that this view is vastly oversimplified and that sense impressions are complex and related to facts through operational rules. Within this network, the scientist plays the key role because he selects and delineates facts, sense impressions, and operational rules. Furthermore, in the search for patterns, it is once again the scientist who performs the search for relationships and tests for validity. Finally, arrangements of simple patterns lead to theories which explain, structure, and predict scientific observations.

Perhaps the importance of individualism in science can best be understood by an analogy of the great French mathematician, Poincare, who said:

> Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house.

To build a house of science requires the best efforts of many individual scientists, each making his contribution in such a way as to create an edifice which is enduring, functional, and esthetically satisfying.

Given the inherent individualism of science, let us now see how it has been manifested and evolved as science has developed.

It was in the Mediterranean region that there was born the first abstract and rational science, which ultimately led to the universal science of our own time. Ancient science was linked closely to philosophy. In order to provide information about the nature of man and the universe in which he lived, the ancient Greeks, through such famous individuals as Aristotle, Hippocrates, and Pythagoras, laid the groundwork for physics, biology, mathematics, and medicine. However, the scientific speculation in Greek society was extremely limited, and confined to a leisured class of intellectuals.

With the triumph of Christianity, all intellectual thought in the Western World, including science, came to be expressed in terms of religious dogma, and as time went on, increasingly confined to churchmen. As a result, the study of the physical universe came to be thought of as an aspect of comprehension of God and the buttressing of revelation. Science came to be practiced, if at all, by clerics for the benefit of the church. As in ancient times, whatever scientific thinking took place was limited to a few individuals in a special class. Because there was little incentive for these individuals to engage in science, and because it was essentially sparetime work, medieval science lapsed into obscure pedantry. In effect, it was the final, rather than the beginning, phase of an intellectual movement which began with Hellenistic science and vanished with the feudal system.

The continuing importance of the individual is emphasized by the fact that when we seek to date the beginning of modern science we often do so with the life and work of Galileo. We do so not only because of the importance of his own scientific discoveries, but because he was the first to apply the scientific method, in the sense that we understand it today.

Of course, the genius of Galileo could not have flowered without the social, political, cultural, and economic ferments which were taking place towards the end of the Middle Ages. There was the invention of printing and the development of towns, trade, and industry. The transformation of the feudalistic social order, although slow and uneven, into a more open, secular society also created the environment for individuals to make their scientific discoveries. And so we have Copernicus, Kepler, and Galileo revealing facts about the heavens; William Gilbert investigating the mariner's compass and revealing what he called "that great magnet, the earth"; and William Harvey publishing his proof of the circulation of blood. In all, it was an age of insurgent ideas, rebelling against the so-called "science" of two thousand years.

In noting the contributions of these great individuals, we may think

of them as "voyaging through strange seas of thought, alone." But this isn't the case at all and one must also emphasize the interrelationships among them and the cumulative growth of scientific knowledge. For example, although Copernicus laid the groundwork for a revolution in scientific thought and Gilbert showed how experimental and inductive methods could add to scientific knowledge, it was Galileo who brought the practical test of the telescope to Copernican astronomy, and combined mathematical deduction with the methods of Gilbert in order to establish the true method of physical science.

In a similar fashion, the English physician, William Harvey, proved that the blood circulates by drawing upon the work of the Italian anatomists and hydraulic engineers under whom he had studied in Padua. Using this education, he proved his revolutionary theory by experimental evidence and deductive reasoning rather than reliance upon Aristotelian dogma. Proof of the mechanism of transport of blood from the arteries to the veins had to wait until the technological development of the microscope, and Malpighi's use of it to demonstrate the existence of capillaries.

By the end of the 17th century, science had definitely arrived. It was becoming increasingly clear to individual scientists that they depended, not only upon the work of their predecessors, but also that of their contemporaries. In various fashions from the 17th century onward, they began to grope toward a structured pooling of their interests and abilities.

Because of the rigid traditionalist structure of the British universities, scientists were precluded from carrying on their work within those institutions. However, men like Boyle, Halley, Gilbert and Wren met regularly at Gresham College in London even during the tumultuous years of Cromwell's rule, forming with their "invisible college" the nucleus of what was to become, with the restoration of Charles II in the 1660's, the Royal Society, which was to form a home for the future plant physiologist, Nehemiah Grew.

Following the Royal Society's lead, provincial societies began to spring up across Britain. Simultaneously, national specialist societies were formed, such as the Linnaean Society in 1788, the Geological Society in 1807, and the Chemical Society in 1840.

In France, meanwhile, the Revolution took the lives of several of her greatest scientists, notably that of Lavoisier. However, the breakup of society paved the way for the establishment of a nationally supported center for scientific research and teaching. The new government established the École Polytechnique in 1794 under the leadership of Lagrange, Laplace, Berthollet, and Haüy. In the following decades, the École flourished, not only through its assemblage of brilliant scholars, but through its successful coupling of research and teaching. Under the patronage of Napoleon, it produced men such as Fresnel, Gay Lussac, and Thenard.

Although England and France were the first countries to establish scientific societies or teaching centers of repute, it was Germany that became pre-eminent in absorbing science into regular university life. Justus von Liebig, a student of Gay Lussac, imported the latter's theories of systematic teaching of chemistry to Germany in 1826. His establishment of a highly successful teaching laboratory was followed rapidly by similar centers at the Universities of Berlin, Gottingen, and others. The transition, however, from the individual work to the modern university laboratory was not complete. For instance, despite the rapid rise of the universities, it was the individual professors, such as Helmholz and Bunsen, who attracted students.

Having established and proven the scientific method and created a medium through the Academies, Societies, and universities for scientists to create and transmit knowledge, the great mosaic of science was filled in rapidly by scientists such as Darwin, Pasteur, Maxwell, and Mendeleev during the formative centuries of the modern world. This pattern of scientific progress through individuals and their institutions was also repeated in the New World, i.e., the United States, though to a much lesser extent initially, because the sanctuary of science still remained in Europe.

As in Europe, science in the new United States evolved from the philosophy of the Enlightenment. Some of America's most influential leaders, like Franklin and Jefferson, were fascinated by natural history and, following the example of Europe, societies were formed such as the American Philosophical Society and the American Academy of Arts and Science. But the members of these societies were not full-time professional scientists, they were mainly men of wealth and position who tended to view research as an interesting pastime. The result was predictable—a severely limited capacity for scientific work, and the deferral of nationally influential Academies until the formation of the National Academy of Sciences during the Civil War.

Fortunately, by the time of the Jacksonian period, increased interest in scientific and technological questions was beginning to develop in the United States. Geological and exploratory surveys were undertaken, and the observatories of the scientific schools at Harvard and Yale were established. Individual scientists like Agassiz, Gray, Silliman, and Dana made noteworthy scientific contributions.

Also, during this period before the Civil War, James Smithson bequeathed to the United States the equivalent of $500,000 to be used "for the increase and diffusion of knowledge among men." Despite considerable opposition to accepting the bequest, it was accepted in 1846, and under the excellent scientific leadership of Joseph Henry, the Smithsonian Institution was established.

However, the overall situation was not satisfactory. College education was still centered on the study of the classics and was based on a rigid curriculum from which no deviation was permitted. Natural science in

this system was not considered important and of little educational value. No laboratory instruction existed in any of these colleges.

As the country developed rapidly, this type of instruction became an absurd anachronism in the great national development from an agricultural to an industrial nation that was beginning to take place. A growing appreciation of the importance of science and technology was developing, and American scientists and engineers were beginning to cross the ocean with the idea of trying to practice here what they were taught in Europe.

Despite these efforts, the various scientific schools did not flourish. The principal cause was lack of funds. Even the Morrill Act of 1862 was initially more important for establishing the principle of Federal aid to agricultural and engineering education than for the funds it provided. While science and science education were limping along in the United States, younger American scientists born during and after the 1840's obtained inspiration from a new source: the German university. For the devotee of scientific investigation, Germany opened up and dramatized the vista of a new goal. The German laboratory and seminar offered these future American professors a novel mode of life, a private mode that turned them aside from the everyday world to the world of research. During the final quarter of the 19th century, few academic Americans believing in the ideal of basic scientific research failed to acknowledge an intellectual debt to an explicitly German style of educational experience. The rigorous, precise examination in German university laboratories of natural phenomena inspired many Americans very deeply. The event which more than any other fixed an indelible image of a research-oriented university was the establishment, in 1876, of the Johns Hopkins University at Baltimore. This institution soon came to symbolize the new ideal of research everywhere in America. The Hopkins atmosphere combined two important qualities: a sense of freedom and at the same time, one of driving dedication. To a certain degree, these qualities have remained present in the leading American graduate schools ever since. For a time, Johns Hopkins stood practically alone as an educational institution dominated by the ideal of scientific research.

The later, more usual pattern was for researchers to form an enclave within a larger university which was mainly devoted to other ends. During the 1890's, graduate schools began to develop a semi-autonomous existence at a number of American campuses, most notably Harvard, Columbia, Chicago, and Wisconsin. The growth of graduate training in these institutions was of great future importance. Research ultimately throve in an even more luxuriant fashion at these larger universities because they could offer a broader and more dependable basis for its existence.

Despite the gradual rise in scientific capabilities and the stimulus of World War I, only a modest amount of academic research was underway at American universities in the early 1920's. Harvard, Yale, Princeton,

Cornell, Columbia, and Hopkins were the leaders in the East; and the Universities of Wisconsin, Michigan, and Chicago, and to a lesser degree, a few others were doing appreciable research in the mid-West. The development of great scientific institutions on the West Coast followed somewhat later. By the mid-1930's, under the leadership of Robert Millikan, a Nobel prize winner in physics, an outstanding group of people were attracted to the faculty at the California Institute of Technology. This institution became excellent even though small, and in so doing, illustrated the ever-present characteristic of intellectual activity that excellence attracts excellence. Similarly, the University of California at Berkeley was comparatively undistinguished until it attracted people like G. N. Lewis, the distinguished chemist, and Ernest O. Lawrence, the originator of the cyclotron. Now Berkeley is renowned in virtually every field of learning. Capable scientists were also attracted to Stanford. Thus, the West Coast moved along well not only in science but in overall academic development.

Notwithstanding the Depression, the forward movement of academic science in the United States was maintained through the 1930's. This forward movement depended almost entirely on the initiative and dedication of devoted men who made do with very little indeed. Many times they went into the machine shops and glass-blowing rooms to make their own equipment—often at their own expense. Their teaching loads were very heavy and yet they spent many long hours in research. Somehow on their very low salaries they were able to attend scientific meetings and visit other laboratories, relying on personal funds. The efforts of these people and encouragement and support of university leaders achieved remarkable results. When World War II broke out, science, though far smaller in size than it is today, was a vigorous, thriving, and excellent enterprise in many institutions throughout the country.

In passing, I think it is important to note that the first-class scientific capabilities that were in being, or almost so, in the U.S., were created by devoted individuals long before the present large volume of Federal funds became available for support of academic institutions. Despite the belief of some, the centers of excellence we speak of today did not become great because of Federal support. They became great on the basis of the leadership of great men, their ideas, and innovations.

These resources proved to be of inestimable value to the war effort. For example, the proximity fuse laboratory was based at Johns Hopkins, the principal radar laboratory at MIT, the electronic countermeasure laboratory at Harvard, and the basic developments of nuclear weapons took place at the Universities of Chicago, Columbia, and California.

Although this wartime activity was new to the universities, and not really compatible with the ideals of an educational institution primarily concerned with education and basic research, they took on the various jobs, and, as you all know, did them extraordinarily well. As it hap-

pened, important advantages also accrued to the universities as a result of their patriotic action.

The wartime experience led to general recognition by the public and its political leaders of the importance of science and its applications to the general welfare and of the important role played by academic institutions in research as well as in the education of future scientists. These, in turn, led to important postwar actions on the part of the Federal Government, among them the determination by the various agencies dependent upon technical progress to support research, and the decision to create the National Science Foundation. The various mission-oriented agencies turned to the universities for large components of their research programs, and the National Science Foundation, by the nature of its objectives, gave most of its support to the academic institutions. Thus, the successful wartime programs conducted by the leading universities and by individual scientists from all academic institutions paved the way for increasingly substantial support in the postwar era to the benefit of all. Initially, research support was mostly carried out through the project method in which individual scientists or small groups of scientists were specifically supported through grants to their institutions. As time went on, these programs grew, and to them were added substantial amounts of support for many aspects of science education directed at producing more and better scientists and engineers. That American science has flourished spectacularly is certainly due in substantial part to these programs.

To give you some idea of the growth in the dimensions of science in the United States, let me describe briefly the growth in scientific and technical manpower since the early or mid-1950's. At the present time, about 190,000 bachelor degrees in science and engineering are being awarded per year. This is about double the number of awards made in the mid-1950's. And yet, from the mid-1950's until the present, the 22-year-old population had increased by only about a third. During the same period of time, the rate of awards of doctorates rose more than two and one-half times from 5,200 to nearly 13,000 per year. This is especially noteworthy, since the relevant population (25-34-year-olds who earn 70 per cent of the doctorates) actually declined about 10 per cent by 1965, and only now has regained its mid-1950 levels. A similar growth is shown in the rise in scientific and engineering employment. While total non-agricultural employment grew by about 30 per cent, science and engineering employment nearly doubled to more than 1.5 million today.

Striking as these figures are, however, they are but extensions of trends that have existed for many decades. For example, the percentage rate of increase in Ph.D. production rates has varied only transitorily throughout this century. What is different is, of course, the absolute magnitude, which has increased exponentially.

The resources placed at the disposal of the scientists, especially by

the Federal Government have changed dramatically. Between 1940 and 1969 Federal support has grown from about $75 million for research *and* development to about $2 billion for basic research alone—of which more than half is used by academic scientists.

Scientists now pursue their work far more rapidly and effectively—both because they have available greater quantities of facilities and services and because many complex and costly equipments have become available that permit far greater extensions of laboratory methodology than would otherwise be feasible or even possible.

The Unity of Science

As scientists have congregated in diverse institutions, the organization of science itself has gone through two successive phases, first of divergence, and now of convergence. In ancient times there were no disciplines or specialists. Science was known as natural philosophy. Intelligent men observed, and attempted to interpret nature in whatever aspect their fancies took them at the moment. As time went on and knowledge grew in quantity and complexity, however, there began to be a fractionation. Although some giants who were well ahead of their time—like Newton, for example—formulated certain laws of nature with elegant simplicity, in general the lack of understanding of universally applicable laws, indeed even of the systematic structure of matter, made it difficult if not impossible to recognize the unity of nature. The various fields of science went their separate ways. There was for a long period little intercourse between the physical and the biological sciences or, indeed, between, say, physics and chemistry. Many disciplines were largely descriptive in nature and concerned with classification.

Recently this trend has been reversed, largely because of the development of more abstract concepts and generally applicable principles and laws. Recognition of such general laws as thermodynamics, electrodynamics, and the quantum theory, going hand in hand with the discovery of the atomicity of matter and the elegant arrangement of the periodic table of the elements, together with our ever-improving techniques for exploring and understanding the structure and behavior of crystals, of cells, of molecules, of atoms, and now even atomic nuclei, have tended to bring the divergent fields together. Examples are innumerable. Discovery of the nature of the atom with its central nucleus and surrounding electron cloud led to the development of quantum electrodynamics, which in turn explained the arrangement of atoms into molecules—of vital significance to the chemists—and into solid structures—of vital interest to the metallurgists. The laws of chemistry have in turn been extended to explain the behavior of the living cell and thence of assemblies of cells into complex organisms. Knowledge of physics, chemistry, and biology have been combined to lead to understanding of the larger and more complex aspects of our environment, be they the solid earth, the oceans, or the atmosphere.

Perhaps the most striking example of all is the way in which one of the oldest sciences, astronomy, and one of the newest, nuclear physics —representing as they do cosmological studies on the one hand, and those of the most microscopic particles on the other—have been combined in astrophysics. Laboratory studies of nuclear reactions led to an understanding of the general mechanisms by which the stars, including our own sun, release their fantastic energies. In turn, studies of stellar phenomena, most recently quasars and pulsars, tell us that we are far from understanding all the processes—and that there may well be types of nuclear reactions of which we have not even dreamed.

Progress along all these and many other lines has led us to recognize that there *is* a universal unity in nature—that her laws are equally applicable to all things, living or inert, and that there is in reality only one science—the understanding of nature in all of her complexity and grandeur.

That science is becoming multidisciplinary with a tendency toward simpler and generally applicable concepts has tended to reinforce the importance of universities, industrial and governmental laboratories, experimental stations and other institutions concerned with providing a meeting ground for scientists and developing and applying knowledge.

Science and Technology

Similar phases, of divergence and then convergence, have existed between science and technology. Both are very old. In very early times they had close relationships; science was then largely observational in character and the same observations, in which anyone could share, served to lead toward both greater understanding and more effective use of natural phenomena. Later, as science delved deeper and tended to become more abstract, and its practitioners more specialized, they and the technologists drew apart and for a long period tended to lead separate existences.

More recently as science began to mature into a complex with greater coordination and institutionalization, the trend reversed; science and the diverse fields of technology began to be mutually reinforcing. In any event, I now will trace briefly this movement toward a unified continuum.

If we begin, as we did before with Galileo, we can detect the inherent interplay between science and technology—the potential of which was not, however, fully realized until the 20th century. For example, technology, which was largely a craft tradition, had contrived a suction pump for terrace agriculture, but it would not raise the water more than 32 feet. Galileo observed this phenomena but it took his student, Torricelli, to conclude that air had weight and to invent the barometer.

This interesting story in which science and technology interacted so fruitfully should not, however, be thought of as representative of the

main currents of the time. Actually, there was comparatively little interplay between them, and what there was tended to be in the direction of technology influencing science by the problems that it posed. For example, the compass stimulated scientific investigations into magnetic phenomena and Newton was frequently involved in such problems of technology as navigation.

Perhaps the greatest commonality that existed between science and technology was to be found in the changing environment they shared. Thus, the previously mentioned social ferments at the end of the Middle Ages affected and benefitted both.

With the demand for better techniques, and better modes of transport and production resulting from the revival of trade and industry, there was a liberation of human ingenuity into hitherto closed fields. And yet, the achievements of technology during the mid-18th century, such as coke smelting, textile machinery, and chronometers were based on the triumphs of "tinkerers and gadgeteers" who relied little, if at all, on the science then available. In the words of Melvin Kranzberg, "The steam engine did more for thermodynamics than thermodynamics ever did for the steam engine."

The situation began to change in the 19th century, though its full integration had to wait until the 20th century. As was natural, one of the first large-scale organized blendings of research and the pursuit of practical ends took place in agriculture where the joint efforts of the universities proper and the experiment stations combined to improve agricultural technology by combining a better understanding of the nature of live organisms and how they grow, with improvements in methods to take advantage of that understanding. Of this, more later. Another somewhat analogous example has been the development of the university hospital which serves the three-fold ends of research, of teaching, and of patient care.

Also beginning in the 19th century, some talented inventors began to find ways to apply the fundamental findings of science directly to practical objectives. We find an early example of this in 1833 when a young scientist named Joseph Henry, in the course of his research into electricity and magnetism, worked out the scientific principles that made possible the electric telegraph. While Henry continued with his fundamental, scholarly research, Samuel Morse made use of the knowledge in fashioning a model of the telegraph which he demonstrated a few years later. The telephone of Alexander Graham Bell followed as a further development. Science was applied on a more intensive and more variegated scale in the laboratories of Thomas Edison at Menlo Park. Originally this facility was just a barn for trying out inventions, but it evolved to demonstrate the need for continuous experimentation closely related to production.

At first manufacturing technology confined its dependence on science to using the results of research conducted elsewhere, but as time went on, technological organizations began to do research themselves. A few

early basic investigations were stimulated in the textile industry, but the first real such fusion of science with technology took place in the field of chemical research—not in its birthplace, England, but in Germany, where great advances in synthetic dyes took place through the application of scientific research to industrial products and uses. This close relationship of research and application has continued and expanded to this day to embrace all branches of industrial chemistry.

Today, virtually all industries are alive to, and make use of, advances in fundamental science and many, such as the chemical, the electronics, and the pharmaceutical industries have large basic research programs of their own. Whole new industries have been formed on the foundation of even fairly recent advances. We have already alluded to the electrical industry and to the telegraph and telephone all based on the researches of Henry, Faraday, and Oersted. Knowledge of electromagnetic waves, based on the theoretical work of Maxwell and the experiments of Heinrich Hertz, gave us the basis for radio and television, which have been made practical by innumerable researches leading to the vacuum tube, the transistor, and all the other component parts.

Just as technology depends on science, so science relies crucially on technology to provide its requisite tools. Scientists have always depended on others for many of the basic ingredients with which they work. At first these were largely materials such as metal and glass, augmented later by machinery, such as lathes, with which to form them to the necessary shapes. Still more recently, industry has provided sophisticated devices, some originally developed for non-scientific purposes, others developed deliberately as scientific instruments.

Perhaps there is no better example of this mutual interdependence than the transistor. Without a fundamental understanding of the structure of semi-conducting solids—an understanding brought about by thousands of basic experimental and theoretical investigations—the transistor that is now of such importance in numerous applications could not even have been imagined let alone developed. Everyone is familiar with the uses of transistors in radio, television and telephone communications, in missile guidance and so on, but it also has had a profound impact upon science. For example, computers are now, of course, indispensable to the progress of science and, in turn, transistors are indispensable to the modern high-speed computer. Many other uses of transistors are to be found in virtually every laboratory.

With more deliberate intent, industry also now provides sophisticated scientific instruments such as electron microscopes, mass spectrometers, nuclear magnetic resonance devices, and even particle accelerators. One of the greatest impacts of the greatly augmented funds available to science as a result of increasing Federal support in recent years has resulted from the fact that the increased numbers of scientific investigators who were able to procure such devices through the command of greatly augmented resources have made the scientific instrument

industry a really viable one. Items that had previously to be fabricated on a one-of-a-kind basis, usually in the laboratory, are now shelf items that a scientist can draw on for his investigations quickly and comparatively cheaply. Thus, communication between science and technology now takes place on a two-way street.

This development of interdependence between science and technology has led to an increasingly close interrelationship between scientists and engineers—one that was markedly stimulated by the partnership they formed during the war. During that period, the scientists and engineers came to understand and respect each other to a degree far beyond what had hitherto been the case. The engineers found that the advanced knowledge, the ideas and the research-mindedness of scientists were of great value in attacking the practical problems faced by engineers. Conversely, the scientists discovered that engineers were useful not only in developing and designing things of practical use but also in helping the scientists in their own work. This rapport has grown markedly over the intervening years so that now it is commonplace for teams of scientists and engineers to work together in all sorts of endeavors ranging from basic research projects to the most sophisticated large system developments in both the civilian and the military spheres. Everyone is familiar with the latter. As an example of the former, before the war it was extremely rare for an engineer to be associated with the development of particle accelerators. Now every group designing and building such accelerators is staffed predominantly by engineers, the role of the physicists being primarily to guide the project in those directions that will make the accelerator most useful as a research tool.

Both the scientists and engineers have been affected by this change. Many physicists, chemists, metallurgists, and others have themselves become virtual engineers; research conducted by engineers now is often very basic in character, so that there is no longer any sharp dividing line. Indeed, we can generalize. Just as there has been a convergence toward unity within science itself, so there has been a drawing together of science and technology in all fields. There is a continuous spectrum of activities and individual interests from the basic physicist or chemist to the developmental engineer, from the basic biochemist or geneticist to those who directly improve our agricultural methods, and from the same fundamental biologists to the clinical physician.

Summary and Conclusions

In these remarks, I have described three converging trends that have characterized the organized and systematic growth of science. From working as individuals scattered in both time and space, scientists began to cohere in progressively larger and more organized societies and institutions, with ever-closer interrelationships within science and between science and technology.

It is interesting to observe how these trends of dependence on creative, dedicated scientists working first as individuals, then through institutions of various types have been replicated in the evolution of agricultural experiment stations. The agricultural experiment station as it exists in the United States today is the product of a process of change, development, and adaptation which can be traced back indirectly to the foundation of the Highland and Agricultural Society in Scotland in 1784. After many unproductive years of amateur experimentation, it took the initiative of Professor James Johnston to demonstrate that laboratory research could be of benefit to agriculture. Professor Johnston's ideas were transplanted to the United States through the efforts of Benjamin Silliman, Sr., of Yale University, and his protegé, John Pitkin Norton, who studied under Johnston in 1844 and 1845. But even these dedicated individuals were unable to overcome local opposition to "book-farming," and progress had to wait on Samuel Johnson, a student of Professor Norton, to return from studies at a German, State-chartered, agricultural experiment station. Many years elapsed, and Federal support was secured through passage of the Morrill Act, before Samuel Johnson succeeded in his endeavors to establish what has become the Connecticut Agricultural Experiment Station, the first such in America. Today, there are 57 main agricultural stations in the U.S. and Puerto Rico, many of which are organized as autonomous departments in State land-grant universities and colleges of which they are integral parts and in whose schools of agriculture most of their leading staff members hold faculty appointments. The substantive value of these stations is evidenced by their rapidly increasing financial support which has increased more than 70 per cent since 1958, and about tripled since 1954.

In this abbreviated review of the development of agricultural experiment stations, we see not only the influence of scientists in shaping increasingly effective organizations, but we have a fine, perhaps leading, example of the pulling together of varied disciplines and the direct interaction of science with technology. Our understanding of agriculture has progressed from an empirical, cut-and-try approach to a complex interdisciplinary science. Today, advanced sciences such as genetics, plant physiology, and soil chemistry are merged into a basic understanding of the biological processes. Surely, the noteworthy growth of agricultural stations in the past two decades reflects the recognition of the beneficial effects which flow from such efforts as well as the continuing exploitation of the science-technology interaction.

We have come a long way since pioneer times when the lone farmer with axe and rifle formed a viable social unit. His knowledge was sufficient for his task—made simple by his isolation. Today, the farmer functions in a complex economic system, and must adapt to the new scientific and technological environment.

More generally, we have, together with science, moved from a feudal to an industrial society. As the old order passed, and the impact of

science has become greater, we have entered into a knowledge society. In this society, we move away from the immediate, personal, and familiar to more abstract concepts in which the centrality of theoretical knowledge and the codification of knowledge into abstract computer-based systems of symbols has become increasingly decisive.

As a result of this trend, there has been a decline in the importance of manual skills with an increase in needs for skills in analysis and the use of intellectual constructs, especially science. In short, a premium on understanding, rather than knowledge of isolated facts.

Science itself has been affected. It is now much more dependent on and even influences its social, political, and economic environment. Great changes have taken place in public and governmental attitudes toward education and toward intellectual and cultural pursuits in general. Initially, science was supported as a means to achieve practical ends, and science education as a means to fill manpower needs. This rather narrow view has been broadened considerably in recent years. There is wider realization that the practical fruits of science can occur only if the tree that bears them—science itself—is helped to grow and flourish. Also, and of great importance, is the notion that our national well-being requires the highest competence in all fields of human endeavor and that this requires high-quality education for every citizen capable of absorbing it.

And yet, at its very roots, science is still characterized by the importance of the individual, within whose mind are generated the ideas, the forward thinking, the tactics, and the strategy that make science grow. And, as always, the individual is impelled mainly by the craving of his intellect to better understand the how and why, and to share his new found knowledge with his fellow man. The word "eureka" well exemplifies today the thrill of successful discovery as it did when uttered by Archimedes two and one-half millennia ago.

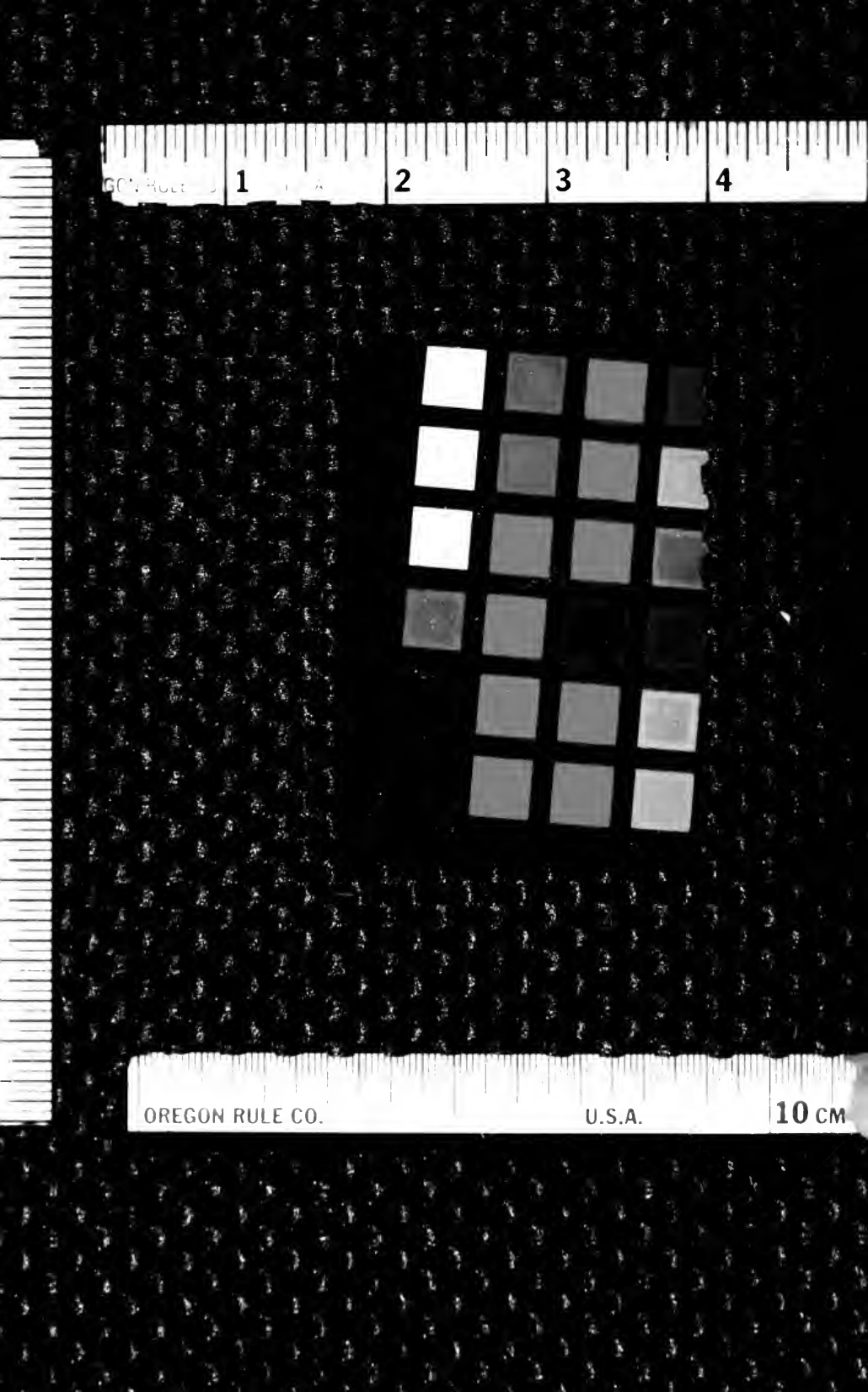
Thus, as we look to the future, our first concern must be to maintain the strength, the freedom, and the integrity of the individual. While accepting the need for and benefits to be derived from bringing the specialized skills of scientists together into an institutional framework, we must be careful not to stifle individual creativeness. While welcoming the establishment of cordial and advantageous relations with technology, we must be careful that the basic characteristics of each are not subverted by the other to the detriment of both. And while we move inexorably toward more general and theoretical formulations of scientific knowledge, we must not lose sight of the need to communicate the flavor and meaning of science to those who are not privileged or able to understand the recondite world of equations, computers, and scientific jargon.

I have no doubt that science will continue to grow in the future as it has in the past, by the accumulation of knowledge through the application of scientific method. In so doing, it will be characterized by the

maintenance of a broad base of inquiry and the exercise of creative imagination together with the utmost objectivity in search and logic. There is every reason to believe that the trends discussed in this lecture will continue into the future. Hopefully, these trends will be tempered so that the maturity of science will be equalled by the responsibility of scientists.

Science will continue to grow in the future because our civilization is irrevocably committed to scientific methods and values. This is both the hope and the challenge for our future.

May 19, 1969


1
2
3
4
OREGON RULE CO.
U.S.A.
10 CM